

ROLE OF VAGINAL SELF-SAMPLING FOR CERVICAL CANCER SCREENING IN WOMEN OF ALIGARH, UTTAR PRADESH, INDIA: A DIAGNOSTIC ACCURACY STUDY

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ABSTRACT

Background: Cervical cancer disproportionately affects women in low and middle-income countries, with India contributing to nearly one-quarter of the global burden, yet screening coverage remains critically low due to barriers including lack of awareness, limited access, and fear of pelvic examinations. **Materials and Methods:** This hospital-based diagnostic accuracy study enrolled 250 sexually active, non-pregnant women aged ≥ 21 years in Western Uttar Pradesh, India, where each participant provided both a self-collected vaginal sample and a clinician-collected cervical sample tested by PCR-based HR-HPV DNA testing (15 genotypes) and liquid-based cytology (LBC), with clinician-collected samples serving as the reference standard. **Results:** Thirteen participants were HR-HPV positive on clinician sampling, of whom 11 were detected by self-sampling too, yielding sensitivity of 84.6%, specificity of 100%, NPV of 99.16%, and excellent agreement ($\kappa=0.91$); overall HR-HPV prevalence was 5.2% (95% CI: 2.8–8.7%) and HPV-16/18 prevalence was 4.0%. On clinician-collected LBC, three women had ASCUS (two HPV-16 positive, one HR-HPV negative) and four had LSIL (one HPV-16, one HPV-18, two HR-HPV negative), while all seven were reported as NILM on self-sampled LBC. **Conclusion:** Vaginal self-sampling demonstrated excellent agreement with clinician sampling for HR-HPV detection and shows potential as a feasible primary screening strategy in resource-limited settings; however, it failed to detect cytological abnormalities, confirming it cannot replace clinician-performed cytology. Future work should focus on integrating HPV self-sampling into existing UP state screening initiatives, strengthening district-level molecular diagnostic infrastructure, and training laboratory technicians in HPV DNA testing.

INTRODUCTION

Cervical cancer is a significant global health issue, especially in developing countries, and is the fourth most common type of cancer in women worldwide after breast cancer, colorectal cancer and lung cancer. Globally, an estimated 660,000 women were diagnosed with cervical cancer in 2022, and 94% of the 350,000 deaths that resulted from it occurred in low and middle-income countries.^[1,2] Although age-standardised incidence rates of cervical cancer have

declined since 1990, India still has one of the highest rates globally and contributes to nearly one-quarter of the total cervical cancer burden worldwide.^[3]

In India, cervical cancer is a major public health problem, accounting for 17% of all female cancer mortality in the age group of 30-69 years and ranks as the second most common cancer in women after breast cancer. India has surpassed China, which held the record for the highest number of cervical cancer cases until 2018 and now shares the sad record of having the largest number of new cases and deaths from cervical cancer in the world 1. As a result, the

lifetime risk of developing cervical cancer is higher in Indian women, where 1 out of every 53 women is likely to be affected compared to 1 out of every 100 women in the more developed world.^[4,5] This underscores the imperative need for efficient cervical cancer screening, HPV vaccination, prevention, and control mechanisms in India which will promote higher compliance of Indian women in general.

The major etiology for cervical cancer is the human papillomavirus (HPV), which is a sexually transmitted disease. Current prevalence of HR HPV infection in the general population in India is around 5 %.^[6] Early diagnosis of HPV infection/cervical cancer and prompt treatment are the only measures that will minimize the morbidity and mortality due to cervical cancer. Traditional cervical cancer screening techniques, including Pap smears, Liquid-based cytology, HPV detection tests and VIA/VILI tend to involve doctors/healthcare providers in taking samples. These techniques are generally invasive and painful. There are several categories of barriers that have been identified that hinder the involvement of women in cervical cancer screening programs. Unawareness, inaccessibility to screening programmes due to geographical constraints, socio-cultural dogmas, embarrassment, shame and fear of the screening process may discourage women from attending cervical cancer screening.^[7-9] Women who have been sexually abused or have been victims of intimate partner violence may be hesitant to accept routine pelvic exams.^[10] Cervical cancer deaths are higher among women who either do not avail themselves of conventional cervical cancer screening programs (non-responders) or have no access to such programs than among women who attend regular screening.^[11]

Earlier research has established that many of the non-responders do undertake screening when provided with the choice of self-sampling for high-risk HPV (HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68) testing.^[12,13] To bridge the gaps in conventional cervical cancer screening methods, self-sampling by women themselves can prove to be a valuable instrument if it provides equivalent accuracy in recognizing cellular alterations and the presence of HR HPV DNA. The concordance and agreement of self-samples for HPV DNA detection were found to be high in similar studies.^[14,15] Cervicovaginal self-collected specimens were found to be as sensitive as physician-collected cervical specimens for the detection of high-risk HPV DNA.^[16-18]

However, there remains a significant gap in the literature from North India, and specifically from Uttar Pradesh (UP), a state that accounts for a disproportionate share of India's under-screened female population. Only 1.5% of the total 27,537,000 women population (aged 30-49 years) of UP had undergone a cervical cancer screening test according to an analysis on NFHS-5 data, of which Aligarh had just 0.2% women who reported cervical cancer screening uptake.^[19] To our knowledge, no prior diagnostic accuracy study from Western UP has

directly compared vaginal self-sampling with clinician-collected sampling using both LBC Pap smear and PCR-based HR-HPV DNA testing in the same participants. This study was therefore designed to address that evidence gap.

The main aims are to compare vaginal self-sampling and clinician sampling (gold standard) based on sensitivity, specificity, predictive values, likelihood ratio, and agreement for detection of cervical cancer and to find the prevalence and typing of HR-HPV among reproductive-aged women in Western Uttar Pradesh.

MATERIALS AND METHODS

A hospital-based diagnostic accuracy study was conducted in the Department of Obstetrics and Gynaecology in collaboration with the Departments of Microbiology, Pathology, and Radiotherapy, at a tertiary care hospital in Aligarh, Uttar Pradesh. The study period extended from March 2024 to February 2026. Ethical approval was obtained through the Institutional ethics committee of our hospital.

Inclusion criteria

Sexually active non-pregnant women aged 21 years or above presenting to the Obstetrics and Gynecology Department were included in the study. Participants had no previous history of cervical cancer and provided consent to participate. Women who had undergone excision or destructive treatment for cervical intraepithelial neoplasia were excluded. Participants were also required not to have used any vaginal medication for at least one week before the examination and not to have had sexual intercourse for at least 48 hours prior to the examination. Additionally, women who were pregnant or experiencing menstrual bleeding at the time of examination were excluded from the study.

Exclusion criteria

Cases of previously diagnosed cervical cancer and women who had received prior therapy for cervical neoplasia were excluded from the study. Women who were not sexually active or had undergone a total hysterectomy were also excluded. Additionally, pregnant women or those within six months postpartum, as well as individuals who were unable to provide informed consent, were not included in the study.

Sample size was calculated using the formula $n = (z^2 P(1-P))/d^2$, where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence and d is precision (corresponding to effect size). Considering P as the prevalence of high-risk HPV infection in India as 5% at 95% CI ($z=1.96$) and d as 3%, our sample size comes out to be approximately 250 ($n \approx 250$).^[6,20]

Total Samples for tests = 500 (Two Smear samples from each woman - 1 Self sampled and 1 Clinician sampled). Eligible women were enrolled following informed consent.

Sampling method: Study participants were recruited using Consecutive sampling from the OPD.

Sample Collection: For clinician-collected sampling, endocervical and exocervical samples were obtained using a BD SurePath™ cytobrush (360° rotation, twice) following speculum insertion. The cytobrush was placed in LBC collection fluid, labelled with sample number and date (no patient name, to minimise bias), and sent to the Cytopathology Department for LBC Pap smear testing.

For vaginal self-sampling, the same participants collected their own samples 48 hours after clinician sampling (to allow cervical cell replenishment). Participants received pictographic instructions and were given a BD SurePath™ cytobrush to self-collect in a private bathroom; researchers remained available outside for clarification. Samples were transferred to BD SurePath™ fluid, labelled identically to clinician samples, and sent to the Cytopathology Department. Laboratory personnel were blinded to clinician sampling results.

All samples were refrigerated in the Department of Pathology. Following satisfactory LBC Pap smear results (evaluated per the Bethesda System), samples were forwarded to the Department of Microbiology for PCR-based HR-HPV DNA testing using the QIAscreen® HPV PCR assay, which detects 15 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68). Results were communicated to participants within 15 days.

Diagnostic performance measures (sensitivity, specificity, PPV, NPV, likelihood ratios) were calculated using clinician-collected samples as the reference standard in STATA SE-64. Kappa agreement was interpreted as: ≤0.20 poor, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 good, and >0.81 excellent. HR-HPV prevalence and genotype distribution were also evaluated.

RESULTS

[Figure 1] is the participant flow diagram. We had 250 participants in our study.

[Table 1] shows the sociodemographic features of our 250 study participants. Most of the participants were in the age group of 29-39 years (36.4%). Mean age of participants was 37.5 years [95%CI = 36.2-38.8 years]. Mean duration of marriage (sexual exposure) was 13 years [95% CI= 12-14.1 years].

Out of the 250 patients tested for HPV-DNA, 13 were reported positive for HR HPV DNA through the samples taken by the clinician. And 11 out of these 13 patients were HR HPV-DNA positive on self-samples as well. [Table 2]

On clinician-collected LBC Pap smear, three women were reported as Atypical squamous cells of undetermined significance (ASCUS) and four as Low grade squamous intraepithelial lesion (LSIL). Among the three ASCUS cases, two were HR-HPV positive (both HPV-16) and one was HR-HPV negative. Among the four LSIL cases, two were HR-HPV positive (one HPV-16, one HPV-18) and two were HR-HPV negative. All seven women with abnormal cytology were reported as NILM on self-sampled LBC. Rest 243 patients reported NILM on both clinician collected LBC pap smear and self-sampled LBC. [Table 3, Table 4]

[Table 5] shows the subtypes of HR HPV DNA detected in HPV positive patients in both clinician and self-sampling. Overall the prevalence of HR HPV infection seen is 5.2% (95% CI= 2.8%-8.7%) while the prevalence of HPV (Type16/18) infection seen is 4 % (95% CI=1.9%-7.2%). HR HPV DNA genotype 16 infection alone in our sample is 2.8% (95% CI=1.1%-5.7%).

Sensitivity of self-sampling for HPV-DNA detection as calculated from [Table 2] is 84.6% (95% CI=54.5%-98.07%) while specificity and PPV are both 100%. Negative predictive value is 99.16% (95%CI=97%-99.8%). Expected agreement is about 90.9% and kappa is 0.91 (excellent agreement).

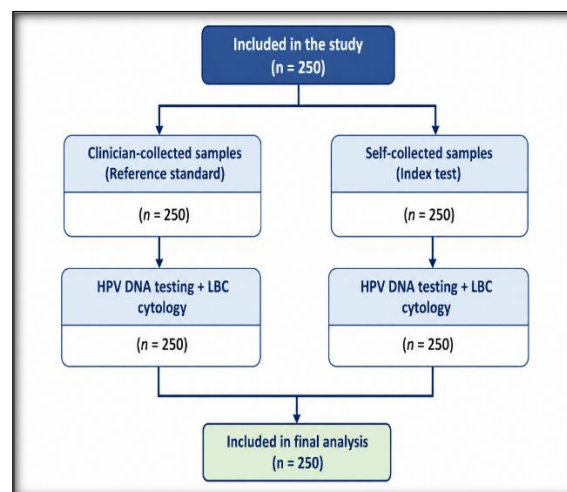


Figure 1: Participant flow diagram

Table 1: Sociodemographic characteristics

Characteristics		n (Total N= 250)
Age group	21-29 years	62
	29-39 years	91
	39-49 years	64
	49-59 years	19
	59 years and above	14
Religion	Islam	132
	Hinduism	115
	Christianity	3
Duration of marriage	0-10 yrs	105
	11-20 yrs	94
	>20 yrs	51

Table 2: 2*2 table for HPV detection

Self-sampling (Index test)	Clinician sampling (Reference standard)		Total
	HPV positive (HPV type 16, HPV type 18, HR HPV, Non HR HPV)	HPV negative	
HPV positive	11	0	11
HPV negative	2	237	239
Total	13	237	250

Table 3: 2*2 table for LBC-Pap smear

Self-sampling (Index test)	Clinician sampling (Reference standard)		Total
	LSIL/ASCUS	NILM	
LSIL/ASCUS	0	0	0
NILM	7	243	250
Total	7	243	250

Table 4: HPV status in abnormal cytology (clinician sampling)

Abnormal status	ASCUS	LSIL	Total
HR HPV 16 positive	2	1	3
HR HPV 18 positive	0	1	1
HR HPV negative	1	2	3
Total	3	4	7

Table 5: HPV genotypes

HPV-DNA Type	Clinician sampling (Reference standard)	Self-sampling (Index test)
HR HPV Type 16-DNA alone	7	5
HR HPV Type 16-DNA + other HPV	0	2
Other HR HPV DNA (not HPV-16/18)	3	2
HR HPV Type 18-DNA	3	2
Total HR-HPV positive	13	11

DISCUSSION

This diagnostic accuracy study evaluated vaginal self-sampling for cervical cancer screening among women attending a tertiary care hospital in Aligarh, Western Uttar Pradesh, a region with limited published data on self-sampling. Vaginal self-sampling demonstrated high sensitivity (84.6%) and perfect specificity (100%) for HR-HPV DNA detection, with near-perfect agreement with clinician-collected specimens ($\kappa = 0.91$), supporting findings from previous studies while providing important regional evidence.

The observed sensitivity and specificity are consistent with systematic reviews and meta-analyses of PCR-based self-sampling, which generally report sensitivity above 70% and kappa values exceeding 0.70.^[16,18] The excellent agreement in our study may reflect the standardized sampling protocol and use of a single HPV detection platform (QIAscreen® PCR). Variations in self-sampling accuracy across studies likely arise from differences in collection and testing methods; while we used cytobrushes, other studies employed tampons, swabs, cervicovaginal brushes, or lavage devices.^[17,21–24]

Two HR-HPV-positive cases identified by clinician sampling were missed on self-sampling, possibly due to sampling variability, lower vaginal viral load, or limited access to the transformation zone.^[16] Nevertheless, the high NPV (99.2%) indicates strong reliability for ruling out HR-HPV infection. Importantly, self-sampling failed to detect any cytological abnormalities; all ASCUS/LSIL cases identified on clinician-collected LBC were reported

as NILM on self-sampled LBC. This confirms that HPV self-sampling and cytology are complementary rather than interchangeable, with clinician sampling remaining essential for cytological assessment.

Although our primary objective was to assess diagnostic accuracy rather than acceptability, most participants were initially hesitant to perform self-sampling. However, after completing the procedure, the majority reported it to be more comfortable and less distressing than clinician-performed sampling. This incidental qualitative observation is consistent with published evidence. De Pauw et al. reported that 93% of 515 women believed self-sampling could improve participation among under-screened populations, and over 95% expressed a favourable attitude after performing the procedure.^[25] Given that the largest proportion of cervical cancer cases in India occur among women who have never been screened, even modest improvements in screening uptake through self-sampling could have substantial public health benefits.

India's cervical cancer screening guidelines recommend screening women aged 30–65 years, primarily using VIA every five years because of its low cost.^[26] Despite more than a decade of VIA implementation, cervical cancer burden remains high, and NFHS-5 reported an overall screening coverage of only 1.9%.^[27] This limited coverage may be attributable to the well-recognized shortcomings of VIA, including subjective interpretation, operator dependence, and poor follow-up systems, particularly in rural settings.^[28]

Improving participation through self-collected HPV testing may help overcome barriers related to sample collection and screening acceptance. Women who are

HPV-positive with normal cytology should undergo repeat Pap smear or liquid-based cytology after one year.^[29,30] Although HPV-DNA testing has higher initial costs, it is considered cost-effective in the long term because early detection reduces the burden of advanced cervical cancer.^[31]

The prevalence of HR-HPV in our study was 5.2% and HPV-16/18 prevalence was 4.0%, both broadly consistent with national estimates of around 5% 6. The slight deviation likely reflects our relatively modest sample size and the hospital-based recruitment setting rather than community-level epidemiology.

Strengths and Limitations

The key strengths of this study include the use of a standardised sampling device and HPV platform for both specimen types, laboratory blinding to collection method, consecutive enrolment to minimise selection bias, and the provision of both LBC and HPV testing from the same specimens allowing a direct comparison of diagnostic performance for two different endpoints. The study also addresses a genuine evidence gap from Western Uttar Pradesh.

Limitations include the relatively small sample size, which limits the precision of sensitivity estimates (wide 95% CI: 54.5–98.1%). The sample size was calculated to estimate the prevalence of HR-HPV infection with adequate precision. A formal sample size calculation for sensitivity estimation was not performed prospectively; however, post-hoc analysis confirms that the observed sensitivity of 84.6% carries wide confidence intervals (54.5–98.1%), reflecting the relatively low number of HR-HPV positive cases, a known limitation of single-centre diagnostic studies in low-prevalence populations. As a single-centre hospital-based study, findings may not be generalisable to the general community population. The 48-hour interval between clinician and self-sampling, though designed to ensure cellular replenishment, introduces the possibility of interval variation in HPV status, though this is unlikely over such a short period. Finally, the study did not formally assess acceptability, which would be a valuable endpoint for future work.

CONCLUSION

Vaginal self-sampling demonstrated near-perfect agreement with clinician-collected cervical sampling for HR-HPV DNA detection, with high sensitivity, specificity, and negative predictive value in women from Aligarh, Western Uttar Pradesh. These findings support its potential role as a feasible primary screening tool for cervical cancer, particularly in resource-limited and geographically underserved settings where clinic-based screening is inaccessible. However, self-sampling cannot replace cytology-based Pap smear screening, as it failed to detect any cytological abnormalities in our cohort.

Future work from this region should focus on: integration of HPV self-sampling into existing UP state cervical cancer screening initiatives; strengthening molecular diagnostic infrastructure at the district level; training of laboratory technicians in HPV DNA testing and quality assurance; and formal evaluation of acceptability and cost-effectiveness in a community-based setting. HPV vaccination of adolescent girls aged 9–14 years should simultaneously be prioritised as the cornerstone of a comprehensive cervical cancer prevention strategy in India.^[32]

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