

Comparison of Visual Inspection with Acetic Acid, Liquid-Based Cytology, and Human Papillomavirus DNA Testing in Cervical Cancer Screening: A Cross-Sectional Study

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Abstract

Introduction: Cervical cancer is the most common gynecological malignancy in Nepal, primarily caused by human papillomavirus infection. The natural history of cervical cancer is well understood, and periodic cervical screening can reduce its incidence by 90–93%. Common screening methods include visual inspection with acetic acid cervical cytology and HPV DNA testing.

Methods: This cross-sectional study was carried out at the outpatient department of Paropakar Maternity and Women's Hospital (PMWH), Thapathali, Kathmandu. The women aged 30–60 years underwent LBC, HPV DNA testing, and VIA and colposcopy guided cervical biopsy. The collected data were entered into Microsoft Excel and analyzed using SPSS version 29. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Results: The sensitivities of LBC, HPV DNA, and VIA were 66%, 66.7%, and 22%, respectively, while the corresponding specificities were 97%, 97.5%, and 95%. The positive predictive values (PPV) of LBC, HPV DNA, and VIA were 57%, 66.7%, and 28%, respectively, while their negative predictive values (NPV) were 97.3%, 98%, and 93%, respectively.

Conclusion: This study concluded that HPV DNA testing and LBC have higher sensitivity and specificity for cervical cancer screening and are therefore recommended.

Keywords: Cervical cancer, HPV DNA, Liquid-based cytology, Screening, VIA

Introduction

Cervical cancer ranks as the fourth most common cancer among women worldwide, with an estimated 604,127 new cases and 341,831 deaths in 2020.¹ It is a leading gynecological cancer in developing countries like Nepal, primarily affecting women in their fifth and sixth decades.² Nepal reports over 10000 new cases each year, with 26,000 to 45,000 women potentially harboring undiagnosed pre-cancerous lesions.³⁻⁵ Up to 90% of cases are preventable through early detection via effective screening methods such as visual inspection with acetic acid (VIA), papanicolaou smear (Pap smear), liquid based cytology (LBC), and human papilloma virus (HPV DNA) testing.²⁻⁵ HPV infection causes 99.7% of cases, making HPV DNA testing the most sensitive and specific screening method.⁶ While VIA is less accurate, it is frequently used in low-resource settings due to its low cost. However, only 5% of women in developing countries undergo regular screening, compared to 85% in developed nations.^{4,6} This study was done with the aim to evaluate the diagnostic accuracy of VIA, LBC, and HPV DNA testing to determine the most suitable method for resource-limited settings.

Methods

This cross-sectional study assessed the diagnostic accuracy of visual inspection with

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acetic acid (VIA), liquid-based cytology (LBC), and HPV DNA testing for cervical cancer screening. The study was conducted at Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, over a one-year period, with data collection spanning six months from January 2023 to June 2023. Married women aged 30–60 years attending the gynecology outpatient department for routine screening were included, as this age group carries a higher risk of cervical cancer. Women with a known diagnosis of cervical carcinoma, those who were pregnant, experiencing active vaginal bleeding, or who declined to provide consent were excluded from the study.

Sample Size was calculated using the formula calculation, $n = Z^2 \times p \times q / d^2$ where $z = 1.96$ (95% confidence interval), $p = 71.4\%$ (sensitivity of VIA), $q = 100 - p$, $d = 8\%$ (maximum tolerable error). This yielded a required sample size of 122. After Institutional Review Board approval, written informed consent was obtained from the participants. Demographic, reproductive, and behavioral data were collected using structured questionnaires. The clinical examination included general physical and pelvic examinations.

Cervical samples for LBC and HPV DNA testing were collected with standardized cervical brushes and Digene cervical samplers (Qiagen, Hilden, Germany). Samples were preserved

in an alcohol-based medium and transported to the laboratory following the manufacturer's instructions. VIA was performed by applying 5% acetic acid to the cervix and inspecting it after one minute under adequate illumination. Acetowhite areas at the squamocolumnar junction were considered positive. Women with both negative and positive results on any screening test underwent colposcopy-guided cervical biopsies. Punch biopsies were performed on suspicious lesions, or four-quadrant biopsies were performed if no visible lesions were present. Tissue specimens were fixed in 10% formalin and sent for histopathological examination; CIN or higher-grade lesions were considered positive. The costs for above mentioned screening tests including LBC, HPV DN and biopsy were supported by hospital administration. Data were entered into SPSS version 29. The sensitivity, specificity, positive predictive value, and negative predictive value for VIA, LBC, and HPV DNA testing were calculated using 2×2 contingency tables. Confidence intervals were calculated where necessary.

Results

A total of 122 women aged 30–60 years participated in this study. Among them, the majority 34 (27.9%) were in the 30–34 age group, followed by 35–39 years 33 (27.0%).

Table 1: Age-wise Distribution of Cytology, VIA, and HPV DNA Results

Age Group (Years)	Total n (%)	Normal Cytology n (%)	Abnormal Cytology n (%)	VIA Positive n (%)	HPV DNA Positive n (%)
30–34	34 (27.9%)	33 (27.0%)	1 (3.0%) (ASCUS)	3 (8.8%)	2 (5.9%)
35–39	33 (27.0%)	31 (25.4%)	1 (3.0%) (ASCUS), 1 (3.0%) (ASC-H)	1 (3.0%)	3 (9.0%)
40–44	22 (18.0%)	21 (17.2%)	1 (4.5%) (HSIL)	1 (4.5%)	0 (0.0%)
45–49	8 (6.5%)	7 (5.7%)	1 (12.5%) (ASCUS)	1 (12.5%)	1 (12.5%)
50–54	10 (8.2%)	9 (7.4%)	1 (10.0%) (HSIL)	1 (10.0%)	3 (30.0%)
55–60	15 (12.3%)	14 (11.5%)	1 (6.7%) (LSIL)	0 (0.0%)	0 (0.0%)
Total	122 (100%)	115 (94.3%)	7 (5.7%)	7 (5.7%)	9 (7.4%)

A total of 115 (94.3%) women had normal cytology results, while 7 (5.7%) showed abnormal findings. These included ASCUS 3 cases, ASC-H 1 case, LSIL 1 case, and HSIL 2 cases. The highest number of abnormalities was found in women aged 35–39 years (two cases), with high-grade lesions (HSIL) identified in women aged 40–44 and 50–54 years. VIA positivity was observed in 7 (5.7%) women, with slightly higher rates in the 30–34 and 50–54 years groups. HPV DNA positivity was detected in 9 (7.4%) women, most prevalent in the 50–54 years group at 30%, followed by 35–39 years at 9.0%. No HPV positivity was found in women aged 40–44 and 55–60 years. Overall, HPV positivity was marginally higher than cytological abnormalities, and some discordance between screening methods was noted, including an HSIL case with a negative HPV test.

Table 2: Correlation between VIA, Cytology, HPV DNA result and histopathology

	Benign	CIN I	CIN II	CIN III	Squamous cell carcinoma	Pearson correlation
VIA positive	5	1	1	0	0	0.439
ASCUS	1	0	2	0	0	0.739
LSIL	0	0	1	0	0	
HSIL	0	0	1	1	0	
ASC-H	0	0	1	0	0	
HPV DNA positive	4	2	2	1	0	0.47

Among VIA positive cases, five were histologically benign, while one case was each corresponding to CIN I, CIN II yielding a moderate positive correlation with histopathology (0.439). For cytology, AUSCUS cases included one benign and two CIN II lesions, showing a strong correlation ($r = 0.793$). LSIL was linked to one CIN II lesion, while HSIL was associated with high grade pathology, including one CIN II and one squamous cell carcinoma. ASC-H cases were linked to one CIN II lesion. Among HPV DNA positive cases, four were benign, two were CIN I, two were CIN II and one was CIN III with moderate correlation ($r = 0.47$).

Table 3: Sensitivity, Specificity, PPV and NPV of VIA

VIA	BIOPSY POSITIVE	BIOPSY NEGATIVE
POSITIVE	2	5
NEGATIVE	7	108

In this study, the sensitivity, specificity, PPV and NPV was 22%, 95%, 28% and 93% respectively.

Table 4: Sensitivity, Specificity, PPV and NPV of LBC report

LBC RESULT	BIOPSY POSITIVE	BIOPSY NEGATIVE
ABNORMAL	4	3
NORMAL	2	113

In this study, the sensitivity, specificity, PPV and NPV was found to be 66.7%, 97.3%, 66.7% and 97.3% respectively.

Table 5: Sensitivity, Specificity, PPV and NPV of HPV DNA result

HPV DNA result	BIOPSY POSITIVE	BIOPSY NEGATIVE
POSITIVE	6	3
NEGATIVE		110

In this study, the sensitivity, specificity, PPV and NPV of HPV DNA result was 66.7%, 97.3%, 66.7% and 97.3% respectively.

Table 6: Comparison of VIA, LBC and HPV DNA results

	Sensitivity	specificity	PPV(positive predictive value)	NPV(negative predictive value)
VIA	22%	95%	28%	93%
LBC	66%	97%	57%	98%
HPV DNA	66.7%	97.3%	66.7%	97.3%

Among the three screening methods, VIA showed low sensitivity (22.2%) but high specificity (95%) with PPV of 28.6%, and NPV was 93.1%. Conversely, both LBC and HPV DNA testing had considerably higher sensitivities at 66.7%, detecting about two-thirds of true cases. LBC's specificity was 97%, with a PPV of 57.1% and NPV of 98.3%. HPV DNA testing had a specificity of 97.3%, PPV of 66.7%, and NPV of 97.3%.

Discussion

This study compared VIA, LBC, and HPV DNA testing among asymptomatic, previously unscreened women, using colposcopy-guided biopsy as the gold standard. Most participants were 30–34 years old, consistent with Nayanai and colleagues, who reported a mean age of 35.74 ± 9.64 years, suggesting that CIN is more prevalent among sexually active women.⁷ These findings support the WHO recommendation to prioritize screening among women aged 35–45 years.⁸ The VIA positivity rate in this study was 5.7%, lower than that reported by Deksissa and G/Mariam (12.9%)⁹ and Vedantham et al (12.7%).¹⁰ This variation could be attributed to factors such as examiner expertise, lower prevalence of cervical lesions, and technical aspects, including the concentration and freshness of acetic acid and lighting conditions. Additionally, VIA in this study was performed by doctors rather than paramedics, which may have contributed to a more specific (95%) but less sensitive (22.2%) outcome compared with the findings of Bhattacharya et al., who reported a sensitivity of 96% and specificity of 62%.¹¹

In contrast, LBC and HPV DNA testing demonstrated higher diagnostic accuracy, with sensitivities of 66.7% and specificities of 97% and 97.3%, respectively. These modalities have proven to be more reliable for detecting cervical intraepithelial lesions and are better suited for tertiary care centers, where laboratory support and cost are less restrictive.

Abnormal cytological reports (ASCUS and above) were observed in 5.7% of cases, aligning with the study by Denny et al (4.4%)¹², Slawson et al (7.1%),¹³ and Basnyat et al (6.8).¹⁴ The distribution of cytological abnormalities in our study included ASCUS (42%), LSIL (14%), HSIL (28%), and ASC-H (14%). In comparison, Tesfaw et al. reported higher LSIL (23.2%) and lower HSIL (4.1%) rates,¹⁵ And studies from the Netherlands and Germany, such as Zhu et al., observed lower LSIL (2%) and HSIL (2.4%) rates.¹⁶ These differences may be attributed to factors such as population risk profile, availability of HPV vaccination, and the strength of screening program structure in developed nations.

Our LBC positivity and ASCUS rates were consistent with those of previous studies by Denny et al,¹² Basnyat et al,¹⁴ and Sankaranarayanan et al.¹⁷ HPV DNA was positive in 7.3% of participants, reinforcing its role as a primary screening tool because of its etiological link to cervical cancer. Although LBC and HPV DNA testing offer better diagnostic accuracy, resource limitations may restrict their use. Despite its lower sensitivity, VIA can still be a valuable method for broadening coverage in underserved areas. A tiered approach to initial VIA screening, followed by LBC or HPV DNA testing in VIA-positive or high-risk women, may enhance early detection while balancing feasibility.

Conclusion

In this cross-sectional study of 122 women undergoing cervical cancer screening, liquid based cytology (LBC) and human papilloma virus (HPV DNA) testing demonstrated substantially

superior diagnostic performance compared to visual inspection with acetic acid (VIA), while evaluated against colposcopy guided histopathology. Although VIA maintained high specificity (95%), its marked low sensitivity (22.2%) limits its utility as a standalone screening modality.

In contrast, both LBC and HPV DNA testing achieved significant higher sensitivity (66.7%) with excellent specificity (>97%). The relatively higher positive predictive value of observed with HPV DNA testing further supports its role as primary screening tool. These findings have important implications for screening strategies in resource limited settings. Overall, the study supports the prioritization of HPV DNA and LBC in screening programme with VIA serving as an adjunct in low resource context, thereby contributing to more effective cervical cancer prevention.

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