ISSN: 3066-8123
Research Article

Science Set Journal of Cancer Research

Accuracy of High-Risk HPV DNA Genotyping, Cytology, and VIA as Triage Strategies for HPV-Positive Women in Cervical Cancer Screening

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Submitted: 05 Juli 2025 **Accepted:** 11 July 2025 **Published:** 18 July 2025

https://doi.org/10.63620/MKSSJCR.2025.1018

Citation: *Thinn, M., Tun, N. M., Mon, H. M., Shein, T. M. M., Soe, H. L., Mon, K. L., & Lwin, H. Y. (2025). Accuracy of high-risk HPV DNA genotyping, cytology, and VIA as triage strategies for HPV-positive women in cervical cancer screening. Sci Set Journal of Cancer Research, 4(4), 01-06.*

Abstract

Introduction: *Human papillomavirus (HPV) testing is increasingly adopted as the primary tool for cervical cancer screening. However, given the transient nature of many HPV infections, effective triage strategies are needed to identify women at true risk of precancerous lesions and to reduce overtreatment.*

Objective: *This study aimed to determine the proportion of abnormal triage results and evaluate the accuracy of HPV genotyping, cytology, and visual inspection with acetic acid (VIA) among HPV-positive women to predict the presence of CIN.*

Methods: *A hospital based diagnostic accuracy study was conducted at Yangon Central Women's Hospital among women aged 25–50 who tested positive for HPV. Participants underwent HPV genotyping, cytology sampling, and VIA. Colposcopy directed biopsy was used as the diagnostic reference standard.*

Results: *Among 169 HPV-positive participants, 34.3% had CIN1+ and 16% had CIN2+ lesions. For CIN2+ detection, cytology (ASCUS threshold) showed 40.7% sensitivity, 57.0% specificity, and 54.4% accuracy. VIA achieved 92.6% sensitivity, 59.9% specificity, and 65.1% accuracy. HPV 16/18 genotyping demonstrated 74.1% sensitivity, 40.1% specificity, and 45.6% accuracy. VIA also showed higher overall predictive accuracy for both CIN1+ and CIN2+ lesions compared to cytology.*

Conclusion: *VIA demonstrated superior sensitivity and predictive accuracy over cytology and HPV genotyping in detecting precancerous lesions among HPV-positive women. As a low-cost and accessible approach, VIA represents an effective triage strategy for low- and middle-income settings, supporting early detection while limiting unnecessary intervention.*

Keywords: Cervical Cancer, HPV, Triage Test

Introduction

The incidence and mortality of cervical cancer has been dropped in various parts of the world, after the knowledge to prevent and detect persistent infection with human papillomavirus (HPV) which is the main cause of this fatal disease. Development of HPV tests, which can detect the nucleic acids of virus is the key achievement in second pillar of cervical cancer prevention to screen the precancerous lesions followed by various treatment

strategies.

Although the cervical cancer burden is dramatically declined in developed world with standardized screening and treatment programme, it continues to constitute a major public health problem in lower middle income countries. It is still the common life threatening cancer among women of the less developed regions so also in Myanmar ranking second after breast [1].

Among various screening methods, Human papillomavirus (HPV) testing as a primary screening tool for cervical cancer has been shown to have better clinical performance, greater sensitivity, and higher reproducibility than standard cytological examination [2, 3].

However, there are some possibilities for positive results without any precancerous lesions related to transient infection in primary HPV screening test. Therefore, different triage tests have been carried out in an attempt to avoid the over treatment issues in the primary HPV testing pathway. Various methods have been used as the triage strategy to predict the need for colposcopy referral as well as presence of precancerous lesions. While cytology and genotyping for high-risk HPV strains are most widely used triage methods, visual assessment using VIA or VILI are proposed to be implemented in low resource settings.

However, the best approach for maximizing programmatic effectiveness is still uncertain and whether these triage strategies are suitable or not to implement in resource constrained areas have yet to be explored properly. In this study, the clinical performance of hrHPV 16/18 genotyping, cytology and VIA tests as the triage in HPV positive women were analysed in an attempt to get more information to set up the reliable resource stratified screening methods.

Material and Methods

It was a hospital based diagnostic accuracy study carried out in cervical cancer screening clinic, Yangon Central Women's Hospital (YCWH). A total of 2183 women aged between 25 to 50 years were screened with careHPV test from January 2019 to April 2020 and 251 women were tested positive. Among them, 169 HPV positive women were recruited for this study.

Cervical cytology specimens were taken with cytobrush from HPV positive women for genotyping and cervical cytology (Pap test) followed by visual inspection with acetic acid (VIA). Subsequently, colposcopy and directed cervical biopsy was performed for histological confirmation, enabling comparison of the diagnostic accuracies of these triage methods.

Results

Table 1: Distribution of Triage Test Results

Triage result	Frequency	Percent
HPV genotyping		
Negative	21	12.4
16 positives	60	35.5
18 positives	45	26.6
Other high-risk group positive	43	25.4
Total	169	100.0
Cytology		
NILM	97	57.4
ASCUS/AGCUS	24	14.20
LSIL	26	15.38
HSIL	19	11.2
Suspicious for cancer	3	1.8
Total	169	100.0
VIA result		
Positive	82	48.5
Negative	87	51.5
Total	169	100.0

HPV genotyping was conducted using the Cobas 4800 system. Among 169 participants who tested positive with the careHPV assay, 21 cases (12.4%) showed negative results upon genotyping. Out of the remaining participants, 60 (35.5%) were positive for HPV 16, 45 (26.6%) for HPV 18, and 43 (25.4%) for other high-risk HPV types. Additionally, six cases exhibited mixed infections: four involving HPV 16 in combination with another high-risk type, and two involving HPV 18 with another high-risk type. These mixed infections were classified under HPV 16 or HPV 18 positive categories for analysis.

Table 2 : Distribution of Histology Results

Histology result	Frequency	Percent
Negative	111	65.7
CIN 1	31	18.3
CIN 2	14	8.3
CIN 3	9	5.3
Ca in situ	3	1.8
Invasive	1	0.6
Total	169	100.0

After the cervical sample collection and VIA test, all HPV positive cases were examined with colposcopy followed by biopsy. Out of 169 hr-HPV-positive cases, CIN1+ lesions were detected in 58(34.3%) women while 27 (16%) women had high grade CIN2 and above lesions.

In the present study, 105 out of 169 CareHPV-positive women found to have HPV types 16 or 18. Among these 105 HPV 16/18-positive cases, 39 women had CIN1 or higher-grade lesions, corresponding to a sensitivity of 67.2%, a specificity of 40.5%, and an overall accuracy of 49.7% for HPV 16/18 genotyping in detecting CIN1+ lesions. As for CIN2+ lesions, 20 of the 27 women with high grade disease were positive for HPV 16 or 18. For the detection of CIN2+ disease, HPV 16/18 genotyping demonstrated a sensitivity of 74.1%, a specificity of 40.1%, and an accuracy of 45.6%.

ASCUS/AGCUS threshold was used for cytology triage and 72women (42.6%) had positive results and remaining 97 women (57.4%) were reported as NILMs. Out of 58 women with histologically confirmed CIN1+ lesions, 32 cases (55.17%) had positive cytology results while 26 (44.82%) were negative for cytology. In the positive cytology group, CIN2+ lesions were detected in 11 women i.e. only 15.27% of cytological abnormality whereas remaining 16 women with CIN2+ lesions were from negative cytology group. For the detection of CIN1+ lesion, cytology triage had 55.2% sensitivity, 64.0% specificity and 60.9% accuracy while for CIN2+ lesion sensitivity, specificity and accuracy were 29.6%, 90.10% and 80.5% respectively.

For the VIA triage, 82 out of 169 women (48.5%) were VIA-positive. Among the VIA positive cases, 45 women (54.78%) were found to have CIN1 or higher-grade lesions, while 25 women (30.48%) had CIN2 or higher-grade lesions. The sensitivity, specificity, and accuracy of VIA for detecting CIN1+ lesions were 77.6%, 66.7%, and 70.4%, respectively. For the detection of CIN2+ lesions, VIA demonstrated a sensitivity of 92.6%, a specificity of 59.9%, and an accuracy of 65.1%.

Table 3: Diagnostic Accuracy of Triage Tests to Identify CIN1+ Lesions in HPV Positive Women

Triage tests	Sensitivity	Specificity	PPV	NPV	Accuracy
Genotyping	67.2%	40.5%	37.1%	70.3%	49.7%
Cytology	55.2%	64.0 %	44.4 %	73.2 %	60.9%
VIA	77.6%	66.7 %	54.9 %	85.1 %	70.4 %

Table 4: Diagnostic Accuracy of Triage Tests to Identify CIN2+ Lesions in HPV Positive Women

Triage tests	Sensitivity	Specificity	PPV	NPV	Accuracy
Genotyping	74.1%	40.1%	19.0%	89.1%	45.6%
Cytology	29.6%	90.1%	36.45	87.1%	80.5%
VIA	92.6%	59.9 %	30.5%	97.7%	65.1 %
Colposcopy	81.5%	92.3%	66.7 %	96.3%	90.5%

Discussion

The accuracy of diagnostic tests for detecting cervical intraepithelial neoplasia (CIN) in HPV positive women varies depending on the specific test used. In this current study, careHPV test was used as the primary screening test for the women attending cervical cancer screening clinic of Yangon Central Women's Hospital, Myanmar. This study population takes into account women aged 25 to 50 even though the hospital policy covers 25-65 years old women. This is because one of the triage is VIA which is not suitable for post-menopausal women [4].

The careHPV™ Test technology is an in vitro nucleic acid hybridization assay with signal amplification for the qualitative detection of 14 high risk HPV types. The HPV types detected by the test are the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

It is developed for use in low-resource settings which is simple, rapid, and affordable. The test can process up to 90 specimens in approximately 2.5 hours and delivers clear positive or negative results for one or more out of these 14 strains, making it suitable for large-scale screening programs. It uses cervical or vaginal

specimens and has good sensitivity for detecting high-risk HPV types.

In the present study, the majority of samples were collected by trained medical personnel, while approximately 20% comprised self-collected vaginal samples. The positive detection rates in self-collected samples were comparable to those collected by doctors or nurses, with an overall positivity rate of 11.49% observed in the total study population of 2,183 women.

In the present study, the accuracy of the triage tests was evaluated not only for the detection of high-grade CIN2+ lesions but also for low-grade CIN1+ lesions since this research work intended for screen, triage and treat approach for hard to reach areas with limited resources. The World Health Organization recommend to treat the HPV positive women without a specific triage test when it is not readily available, typically by destroying the whole transformation zone with thermal coagulation [5].

Hence, if a triage test can detect any degree of precancerous lesion, even low-grade changes can be treated appropriately, reducing the risk of missed cases. This enables women who cannot attend frequent follow-up visits and wish to be treated even for low-grade lesions to receive timely and more précised treatment in just a few visits.

The effectiveness of cytology as a triage test for detecting precancerous lesions in HPV-positive women, measured by its sensitivity, specificity, and accuracy can vary based on several factors, such as sample quality, the presence of additional risk factors, and the expertise of the healthcare provider interpreting the results.

VIA is a low-cost, point-of-care test which can offer readily available results with moderate sensitivity (typically 50–80%) and variable specificity, making it useful in low-resource settings but prone to observer variation and false positives results [6, 7]. However, intensive training is an essential key to maximize its effectiveness. On the other hand, HPV genotyping, particularly for high-risk types like HPV 16 and 18, offers higher validity in predicting CIN2+ lesions. Studies show HPV 16/18 genotyping alone can achieve sensitivities around 60–80% with improved specificity over VIA [8, 9]. While VIA remains accessible and feasible where laboratory infrastructure is limited, HPV genotyping provides better risk stratification.

In the current study, for the detection of CIN1+ lesions among HPV-positive women, the VIA test demonstrated a higher sensitivity (77.6%) compared to HPV 16/18 genotyping (67.2%) and cytology (55.2%). Additionally, VIA showed slightly higher specificity (66.7%) than cytology (64.0%) and considerably higher specificity than HPV 16/18 genotyping (40.5%). Overall, both HPV genotyping and VIA demonstrated relatively comparable performance as triage tests for predicting CIN1+ disease, outperforming cytology in this context. However, HPV genotyping exhibited a lower specificity, as the mere presence of the virus does not necessarily indicate the existence of precancerous lesions.

To predict the presence of CIN2+ lesions in HPV positive women, sensitivity of VIA triage and HPV genotyping had much higher sensitivities than cytology i.e., 92.6% and 74.1% while it was only 29.6% for cytology. However, HPV 16/18 genotyping had a lower specificity of 40.1% compared to cytology and VIA triages. The results of this study showed that VIA triage had a high predictive accuracy for both CIN1+ and CIN2+ lesions i.e. 70.40% and 65.1% respectively.

Sensitivity of the VIA triage is significantly higher than cytology for prediction of both CIN1+ and CIN2+ lesions. Although the specificity of cytology triage in this study was quite high which showed as 90.1% with 80.5% accuracy, it had the sensitivity of only 29.6% to detect CIN2+ disease.

On the other hand, specificity of liquid based cytology for CIN2+ lesions revealed as 90.1%which was higher than HPV16/18 genotyping i.e.40.1%. These findings regarding the cytology triage denoted that its ability to detect the high grade precancerous lesion of cervix is much lower than the other tests even though the specificity is higher making the test less reliable while the test related resources are the major problem in developing countries.

According to the FRIDA study conducted by, a large-scale investigation carried out in Mexico between 2013 and 2016, the performance of liquid-based cytology as a triage method for HPV-positive women demonstrated a sensitivity of 42.9% (95% CI, 34.1%–52.0%). This sensitivity was lower than that observed for HPV-16 genotyping alone (51.6%; 95% CI, 42.5%–60.6%) and for combined HPV-16/18 genotyping (58.3%; 95% CI, 49.1%–67.0%) in detecting cervical intraepithelial neoplasia grade 2 or higher. The sensitivity of cytology of the present study is obviously lower than FRIDA study i.e. 29.6% vs 42.9% while that of genotyping is 74.1% vs 58.3% for prediction of high-grade lesions [10].

The present study also analysed the accuracy of the colposcopy for high grade lesions where Swede score of ≥5 was regarded as high grade lesion. Not surprisingly, it showed highest accuracy of 90.5% while sensitivity of 81.5%% and 92.3% specificity for detection of CIN 2+ disease. Therefore, colposcopy is superior to other triage method to identify the presence of precancerous lesions in HPV based screening programs.

However, both primary HPV testing and associated different triage strategies including colposcopy could remain as particular challenges for low and low middle income countries. The lack of cytologists, colposcopists, pathologists and associated quality assurance usually means cytology or colposcopy as a triage method cannot be contemplated especially in resource constraint areas. As of HPV genotyping triage, financial limitation as well as inadequate laboratory and colposcopy facilities make it difficult to implement in the developing world.

The ESTAMPA study conducted in five Latin American study centers, enrolled 25,628 participants and 3612women (14%) of them tested positive for HPV. Of those positive women, 3204 (89%) underwent VIA as a triage test, followed by colposcopy and histological assessment. This process led to the identification of 229 cases of CIN2 (7.3%) and 328 cases of CIN3+ (10.4%). The study evaluated the performance of VIA as a triage method for HPV-positive women and reported a sensitivity of 82.0% (95% CI: 78.6–85.0) for detecting high-grade CIN2+ lesions

in women under 50 years of age [11]. The results of the current study revealed a higher VIA sensitivity of 92.6% to predict CIN2+ disease which might be due to the fact that our research work was a small scale study performed in a tertiary center while most of the observers were trained gynecologists, highlighting the importance of rigorous training for VIA test.

In a study from Cameroon conducted between 2018 and 2020, 294 women out of 1,582 tested (18.6%) found to have HPV infection. These women were triaged using VIA, cytology, and HPV genotyping. CIN2+ lesions were found in 12.2% of cases. The sensitivities and specificities for detecting CIN2+ were 77.1% and 57.4%for VIA, 80.0% and 76.7%for cytology and 60% and near 65% for HPV16/18/45 genotyping [12].

In comparison, the present study reported 11. 49% HPV positive rate with much higher VIA sensitivity (92.6%) but lower specificity (59.9%), while HPV genotyping had higher sensitivity (74.1%) but lower specificity (40.1%), and cytology showed an un acceptable sensitivity (29.6%) but excellent specificity (90.1%). However, those studies consistently demonstrate the trade-offs between sensitivity and specificity across triage methods, with VIA providing strong sensitivity and supporting its role in HPV-positive triage, especially where diagnostic resources are limited.
Although the performance of visual inspection method to detect the high-grade lesions is variable in different studies for HPV positive women, many of them reported high sensitivities of more than 80% provided that the examiners got intensive trainings [13].

Based on the comparative data from multiple studies, visual inspection with acetic acid (VIA) appears to be a preferable triage method for HPV-positive women. Although the specificity of VIA may be lower than that of cytology or HPV genotyping, its consistently high sensitivity for the detection of CIN2+ lesions highlights its effectiveness in identifying clinically significant high grade precancerous lesions of the cervix. On account of the affordability, simplicity with immediate results and ease of implementation, VIA remains particularly suitable for use in low-resource settings where access to cytology or molecular testing may be limited.

Conclusion

In conclusion, this study supports the use of VIA as a practical triage method for HPV-positive women in low-resource settings with its reliable accuracy for detecting both CIN1+ and CIN2+ lesions. It clearly illustrates that VIA, despite being prone to observer variability, maintains high sensitivity which is critical for minimizing missed high-grade lesions in settings where follow-up is challenging. Additionally, this study also points out that the significance of proper training and quality assurance programs are crucial to ensure the accurate interpretation of VIA results and the appropriate management of women with abnormal findings [14].

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