Article published online: 2024-12-11

Cervical Cancer Screening: Comparing PAPs Smear with VIA/VILI in Semiurban Women of Delhi

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Ind J Med Paediatr Oncol 2025;46:200–206.

Abstract

Introduction Screening with PAPs smear for screening of cervical cancer has been the gold standard for many years in high-income countries but has still not been successful in low- and middle-income country like India due to unavailability at public ground level. Thus, a simple, effective, and low-cost alternative to cervical cytology for cervical cancer prevention is urgently needed for high-risk, low-resource settings.

Objectives The aim was to compare the efficacy of visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) with the PAP smear for screening precursor intraepithelial lesions and early signs of cervical cancer, and to evaluate their potential as alternative screening methods to the PAP smear in a semiurban population in Delhi.

Materials and Methods A total of 127 married, nonpregnant patients between 18 and 65 years were randomly selected from gynecology outpatient department. Then, PAP smear samples were taken from all patients followed by VIA and VILI. Biopsies were then taken from those who showed positive findings in either of the screening methods. Out of these, 50 PAP and VIA-negative women were included as a control group. The diagnostic accuracy of VIA and VILI was compared with PAP smear, for diagnosis of precancerous lesions.

Results VIA and VILI results were positive in 22 patients out of 127 (17.3%). PAP cytology report showed epithelial cell abnormality in 13 cases (10.2%) with atypical squamous cells of undetermined significance in 1 case, atypical glandular cells of undetermined significance in 4 cases, low-grade squamous intraepithelial lesion in 3 cases, and high-grade squamous intraepithelial lesions in 5 cases. PAP smear had showed better specificity (90.79%) and less sensitivity (85.71%) as compared with VIA and VILI method. Sensitivity of VIA was 100% and specificity was 80.26%. Similar parameters were seen with the VILI method also. The overall *p*-value for all the parameters of either screening method was > 0.05; hence, both methods are comparable for screening.

Conclusion VIA and VILI can be employed as initial screening tools in place of PAP smear, particularly in countries with limited resources or developing regions.

Keywords

- visual inspection by acetic acid (VIA)
- visual inspection by Lugol's iodine (VILI)
- PAP smear
- cancer cervix
- screening
- low- and middle-income countries (LMIC)

article published online
December 11, 2024

DOI https://doi.org/10.1055/s-0044-1800949.
ISSN 0971-5851.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

Carcinoma cervix is the fourth most common cancer among women worldwide, while in low- and middle-income countries (LMICs) it ranks second in gynecological cancers, with approximately 660,000 new cases and 350,000 deaths globally in 2022.[1] GLOBOCAN also estimated an age-standardized incidence rate of 18 per 100,000 women and a cumulative risk of 2.01%.[2] The cervical cancer occurs as a result of long-term persisting infection with high-risk human papillomavirus (HPV), which is accountable for 90 to 100% of cervical cancer cases. Out of which high-risk type 16 and 18 are the two most common, and constitute for about 70% of carcinoma cervix cases.[3]

High-risk behaviors are poor genital hygiene, early age at marriage, early age at first sexual intercourse, multiple partners, repeated pregnancies, long-term contraceptive use particularly oral contraceptives, smoking, and multiparity, which increase the risk of acquiring HPV in women.[4] Though deadliest, yet it is one cancer which is preventable if an effective treatment is given at an earliest stage, which is easily detectable by routine screening. There are approximately 272.8 million women eligible for screening in India.[5] Numerous efforts are in progress for the prevention of cervical cancer by both the government and nonprofit organizations since long but are yet to make any meaningful impact. The screening methods employed by most advanced countries include PAP smear, HPV deoxyribonucleic acid testing, visual inspection with acetic acid (VIA), or a combination of all these. The United States and other high-income countries have reported a reduction in number of new cases of carcinoma cervix by routine screening with PAP smear in sexually active women.[6] The implementation of PAP smears in low resource settings like India is limited due to inadequate health care infrastructure, restricted health budgets, and a shortage of laboratories and specialized personnel needed for slide preparation and diagnosis. Repeat PAPs smear is needed once every 3 years due to its lower sensitivity. Technical and financial limitations in organizing cytology screenings have prompted the evaluation of visual inspection methods as viable alternatives.

HPV testing is also recommended as an alternative for screening, but it is associated with high costs. Thus, visual inspection methods like VIA and visual inspection with Lugol's iodine (VILI) are the low-cost screening tools that have been proposed as alternatives to PAP smear-based programs, with VIA being the most commonly employed and researched. VIA is simple, cost-effective, and easily scalable for large populations. It requires no laboratory support and can be reliably performed by trained paramedical staff, nurses, and doctors. However, the gold standard for diagnosis of cervical dysplasia is colposcopy-guided biopsy but it is more time-consuming and requires handling by a specialist with good experience.[7] Thus, in this study, it is hypothesized that visual inspection methods have similar screening efficacy as compared with PAPs, and hence they can replace PAPs for mass screening. They have the potential to be utilized as an alternate first screening method and be a pragmatic and effective public health approach for cervical cancer prevention.

Materials and Methods

Study Design

This is a cross-sectional study, carried out at the Department of Obstetrics and Gynecology, Kasturba Hospital, Delhi, India, after due clearance from the institutional ethical committee.

Sample Size

A total of 127 patients were selected from the gynecology outpatient department (OPD) and enrolled for the study after informed consent. The sample size was calculated as per the study by Das et al,[8] using the formula:

$$N = \frac{Z\alpha\text{^}2 \times [P\,(1-P)]}{L\text{^}2}$$

The minimum sample size was around 102.67, nearly $= 103$, taking $Z\alpha$ at a confidence level of 95% as 1.96. L as margin of error $= 5\%$. P is the prevalence of preinvasive lesion of cervical cancer being 7.2%.[9]

Inclusion Criterion

Only married, nonpregnant women between the age group of 18 and 65 years were included.

Exclusion Criterion

Unmarried women, women with confirmed or suspected pregnancy, those having active bleeding per vaginal, previously diagnosed and treated cases of cervical intraepithelial lesion or carcinoma cervix, posthysterectomy patients, and females with major degree of uterine prolapse were excluded from the present study.

Ethics

The institutional ethics committee approved the study, number: D /880/KH/2022 dated August 30, 2022. All participants were explained about the purpose of the study in detail. Consent was taken for participation and all the procedures. Their participation in the study was completely voluntary with the option to withdraw anytime during study. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration of 1964, as revised in 2000.

Most women in this hospital-based study are from semi-urban areas of Delhi, presenting to the gynecological OPD with various complaints such as vaginal discharge, abnormal bleeding, and other related issues. A detailed pro forma was filled including their socioeconomic status and other demographic details, followed by proper clinical examination. PAP smear samples were taken from all patients followed by VIA and VILI.

VIA was performed by applying 3 to 5% acetic acid on to the ectocervix, findings were observed after a gap of

60 seconds. The test results were reported as VIA positive if clearly demarcated, opaque/dense acetowhite lesions with well-defined or raised margins, in the transformation zone or close to the squamocolumnar junction (SCJ), were present. The time taken to appear and disappear was also noted. The location of lesion and area as demarcated by quadrant involvement were also noted. The patients who did not take up white color on acetic acid or showed faint acetowhitening, which disappeared in less than 60 seconds (early to appear, early to disappear), were classified as VIA negative.

VILI was done by Lugol's iodine application to the ectocervix and it was visualized within 30 seconds. The test results were reported as VILI positive if there was presence of bright canary yellow/mustard yellow/saffron yellow areas, without any iodine uptake seen in the transformation zone close to or abutting the SCJ, or when the entire ectocervix seems yellow.

A total 50 VIA/VILI and PAPs negative cases were taken as controls from this group. PAPs smear was reported using the Bethesda System 2014.[10] Biopsy was taken in those who showed positive findings in either of the screening method, and random four-quadrant biopsy was taken from these 50 controls.

Patients were followed up in the OPD for 2 weeks. Further management was done as per the diagnosis established and hospital management protocol. The study outcomes were measured as per the following:

Primary outcome: Diagnostic accuracy of VIA and VILI for screening of preinvasive and early manifestations of carcinoma cervix.

Secondary outcome: Comparison of diagnostic accuracy including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of VIA and VILI with PAPs smear for the detection of intraepithelial lesions, preinvasive, and early manifestations of carcinoma cervix.

Statistical Analysis

The collected data was entered in Microsoft Excel and statistically evaluated using Statistical Package for Social Sciences (SPSS) version 25.0. Sensitivity, specificity, PPV, and NPV of each screening test were calculated. Chi-square test was performed and a *p*-value of less than 0.05 was considered statistically significant.

Results

Majority of the study participants belonged to the age group of 30 to 39 years (40.2%) and lower middle-class category from semiurban areas of Delhi. Note that 50.4% of women in our study group had >2 children. The mean age at coitus was 20.11 ± 3.65 years. Majority of patients presented with pelvic inflammatory symptom (88.9%), and only 8 patients had postcoital bleeding.

Ninety-six patients (75.6%) had negative PAP smear report for intraepithelial lesion or malignancy. Thirteen out of 127 women showed epithelial cell abnormality on PAPs (10.2%), with atypical squamous cells of undetermined significance (ASCUS) in 1 case, atypical glandular cells of undetermined significance (AGUS) in 4 cases, low-grade squamous intraepithelial lesion (LSIL) in 3 cases, and high-grade squamous intraepithelial lesions (HSIL) in 5 cases. All patients with AGUS on PAP report were further evaluated by endocervical curettage and endometrial aspiration biopsy, which came out to be negative in all four cases. These patients were advised for regular follow-up.

Twenty-two women were positive for both VIA and VILI (17.3%). The positive results on VIA and VILI were same in the study participants. Comparing VIA and VILI with PAP, of 105 VIA-negative cases, PAP detected 1 LSIL, 1 ASCUS, and 4 AGUS (►**Table 1**) (►**Fig. 1**).

Cervical biopsies were performed on 78 patients: 28 with positive screening results (either PAP positive or positive on both VIA and VILI) and 50 controls. Histopathology from cervical biopsy was used as the confirmatory test, serving as the gold standard for evaluating the diagnostic efficacy of PAP, VIA, and VILI in detecting preinvasive cervical lesions.

For these 78 patients, sensitivity, specificity, PPV, and NPV were calculated for each diagnostic method (PAP, VIA, and VILI) by constructing 2×2 tables. Groups of subjects were organized according to test results from each method, and outcomes were compared against the histopathology results.

Table 1 Comparison of PAPs and VIA/VILI reports

PAPs	VIA/VILI		
	Negative	Positive	Total
Negative (NILM)	84	12	96
Inflammatory	15	3	18
AGUS	4	0	4
ASCUS	1	0	1
LSIL	1	2	3
HSIL	0	5	5
Total	105	22	127

Abbreviations: AGUS, atypical glandular cells of undetermined significance; ASCUS, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine.

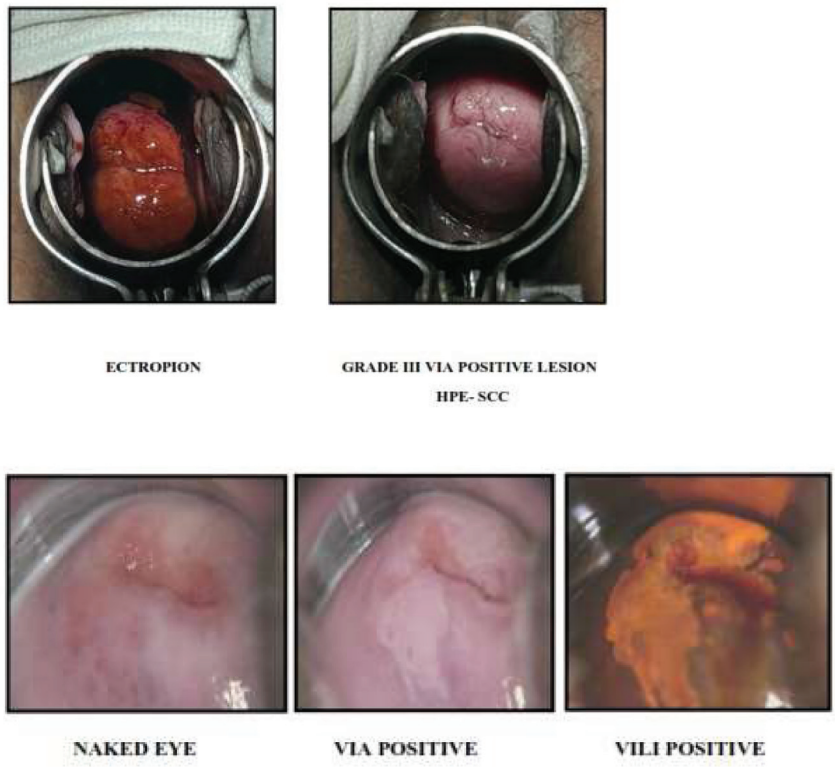

Fig. 1 Visual inspection with acetic acid/visual inspection with Lugol's iodine (VIA/VILI) appearance.

The chi-square test was used to assess diagnostic accuracy, with statistical significance set at a p-value < 0.05.

The data shows that the PAP smear has better specificity (90.79%) as compared with that of VIA (80.26%) and VILI (80.26%), which means PAP has better ability to designate a patient without disease as negative.

Whereas the sensitivity of VIA (100%) and VILI (100%) is much better than that of PAP smear reports (85.71%), that is, they have better ability to designate an individual with disease as positive.

The PPV of PAPs is better (46.15%) than the PPV of VIA (31.82%) and VILI (31.82%), whereas the NPV is better with VIA (100%) and VILI (100%) than that of PAP smear (98.57%).

But the overall p-value of comparisons of diagnostic accuracy of all the three methods, that is, PAPs, VIA, and VILI is > 0.05; hence, the difference is not statistically significant.

Abnormal histopathology was found in seven cases (cervical intraepithelial neoplasia [CIN] grade 1 in two cases and squamous cell carcinoma in five cases), all of which VIA/VILI detected, yielding a 100% NPV. Of 13 PAP-positive cases, only 6 showed abnormal biopsy results. PAP smear showed better specificity (90.79%) but lower sensitivity (85.71%) compared with VIA/VILI, which had 80.26% specificity and 100% sensitivity. The PPV/NPV for PAP were 46.15%/98.57%, and for VIA/VILI, 31.82%/100% (►**Tables 2–4**).

After the calculation of p-value for each of the parameter of each screening method, it has been observed that the p-value of every parameter is > 0.05, hence the differences between diagnostic efficacy of VIA or VILI methods with PAPs method are seen statistically insignificant. Therefore, both screening methodologies are to be considered comparable.

Discussion

Carcinoma cervix is the second most common gynecological cancer in Indian women and is considered preventable. The World Health Organization (WHO) recommends VIA and VILI for the detection of precursor cervical cancer lesions in low resource countries as per screen and treat approach.[10,11] These tests are considered affordable, acceptable, and particularly befitting for the screening in developing nations. The results of this test are readily available, thus permitting the same-day screening and treatment strategy.[12,13]

Table 2 Comparison of VIA/VILI with biopsy ($n = 78$)

Biopsy	VIA/VILI findings		
	Negative	Positive	Total
Negative for CIN/malignancy	44	7	51
Infections	10	7	17
Squamous hyperplasia	2	1	3
CIN 1	0	2	2
SCC	0	5	5
Total	56	22	78

Abbreviations: CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine.

Table 3 Comparison of PAP smear with biopsy ($n = 78$)

Biopsy	PAP smear			
	PAP –ve	PAP inflammatory	PAP +ve	Total
Negative	40	7	4	51
Infections	8	6	3	17
Squamous hyperplasia	3	0	0	3
CIN 1	1	0	1	2
SCC	0	0	5	5
Total	52	13	13	78

Abbreviations: CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma.

This study was conducted to compare the diagnostic efficacy of PAPs smear and visual inspection methods. The PAP smear and visual inspection methods were performed in the same set of patients to compare their results and to evaluate their potential as alternative screening methods to the PAP smear in a semiurban population in Delhi.

It was observed that there were only 18.8% patients who belonged to the age group of > 45 years but showed maximum positivity on PAP smear (29.16%), visual inspection methods (25%), and histopathology report (16.66%), corroborating with the studies done by Bhattacharyya et l[14] and Ami et al[15] who showed maximum positive cases in the age group of > 40 years.

About 17.3% of our screened population showed positive results with VIA and VILI method, which was very much similar with the study done by Das et al[8] who reported 14% VIA positive results and 14% VILI positive results in 200 women. Similarly, the study done by Bhattacharyya et al[14] showed VIA result positivity in 17.3% population but VILI positivity in only 8.6%.

VIA and VILI are cost-effective methods with no extra equipment or laboratory backup requirement. The training and skill for VIA and VILI can be easily imparted to paramedical workers and nurses and hence can be used at a very large scale reaching the remote areas also. They also have the advantage of immediate availability of results, especially if the results are negative; however, a biopsy needs to be performed by a gynecologist if the result is positive. Fokom-Domgue et al[16] also indicated similar results in his meta-analysis for primary screening of cervical cancer in sub-Saharan Africa. VILI was reported to be the most precise substitute to cytology.

There were 13 patients (10.8%) with epithelial cell abnormality on PAP smear in the present study, whereas 14.2% patients had inflammatory report and 96 cases (75.6%) showed no detectable findings. Similar results were reported by Sinha et al[17] where 60.00% showed no major lesions, 31.5% had inflammatory reports, and 8.5% patients showed LSIL or HSIL. Another study by Consul et al[18] reported 72.3% normal smears, 11.00% with inflammatory smears, and 16.27% with positive PAPs smear.

Diagnostic efficacy of PAPs smear was comparable with the one calculated by Consul et al[18] and Bhattacharyya et al[14] as evidenced in a study by Shastri et al.[19] PAPs smear demonstrates moderate sensitivity of 57.4%, while boasting a high specificity of 98.6% in detecting HSIL. Satyanarayana et al[20] observed that the detection rate for CIN I and II by visual inspection methods was more than the PAPs, whereas the detection rate for CIN III was 100% by PAPs smear cytology method. However, sensitivity of PAPs smear (87.5%) for CIN III+ detection was higher than the sensitivity of VIA (50.00%).

However, effective PAP smear screening requires significant infrastructure, including well-trained cytotechnicians, reliable laboratory resources, maintained equipment, logistical arrangements for transport to dependable labs, streamlined communication systems for delivering results, and efficient referral systems for follow-up diagnosis and treatment. Patients often perceive PAP smear as unnecessary and fail to return for follow-up visits to collect cytology reports,

Table 4 Comparison of diagnostic accuracy of screening procedures

	VIA	VILI	PAP smear		–*p*-Value	
				VIA vs. VILI	VIA vs. PAP	VILI vs. PAP
Sensitivity	100%	100%	85.71%	–	1	1
Specificity	80.26%	80.26%	90.79%	–	0.06	0.06
Positive predictive value	31.82%	31.82%	46.15%	–	0.14	0.14
Negative predictive value	100%	100%	98.57%	–	0.98	0.98
Accuracy	81.93%	81.93%	90.36%	–	0.11	0.11

Abbreviations: VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine.

leading to frequent loss to follow-up. Moreover, the financial burden of cytology charges poses a challenge for many. As reported by Juneja et al,[21] estimates suggest that despite significant efforts to expand cytology services in India, screening even a quarter of the population once in a lifetime may remain unattainable.

The diagnostic efficacy calculated in our study of VIA and VILI were very much similar to the studies done by Blumenthal et al,[8] Sankaranarayanan et al,[22] and Bhattacharyya et al.[14] But certain limitations had been recognized in visual inspection method by different studies. According to a study by Carol et al,[23] women with cervicitis or inflamed cervix were found to be twice as likely to test positive by VIA compared with those without any infection (odds ratio: 2.0, 95% confidence interval: 1.0–3.7). These findings suggest that the cervicitis could be a cause of false positive result by VIA. Also, Ngoma et al[24] showed that VIA positivity is highest immediately after training and retraining, thus indicating that the sensitivity of VIA is dependent on training and skills of the practitioner.

Therefore, the limitation of this procedure is the high number of false-positive cases and lower specificity. However, VIA and VILI can serve as a substitute to PAP smear cytology in a country like India, where resources are limited. There are insufficient cytologists, proper infrastructure, or labs, and where mass screening for early detection of precursor lesions of cervical carcinoma is needed for a large population. While the WHO recommends incorporating HPV testing into screening protocols, its limited availability and high cost in LMICs underscore the need for an affordable and accessible alternative screening method that can be implemented at the peripheral level to reach a wider population.

The introduction of noncytological-based screening methods can bring a paradigm shift in cervical cancer screening.

This study also aimed to raise awareness about the risk factors for cervical carcinoma, promote preventive health-seeking behavior, educate about the signs and symptoms of cervical carcinoma, screen for early precursor lesions, and motivate individuals for regular screening until the age of 65. It targets the disease through primary prevention before its onset and employs secondary prevention through effective screening and treatment.

Limitations

Our research indicates that VIA and VILI tests exhibit sufficient accuracy for cervical cancer screening in India, given proper training and quality assurance measures. However, the high accuracy measures obtained for these tests may be misleading, considering the hospital-based study on small number of subjects and the limitations of the gold standard like colposcopy. Additional large-scale population-based studies are required to evaluate the impact of screening strategies employing these tests on the incidence and mortality rates of cervical cancer in the region.

Conclusion

There has been a rise in cervical carcinoma cases, emphasizing the need for widespread screening, especially in resource-limited countries. Visual inspection methods like VIA and VILI are gaining attention in developing regions like India. Our research shows that VIA and VILI have comparable sensitivity (100% vs. 85.71%) and specificity (80.26% vs. 90.79%) to PAP smears (p-value > 0.05), making them a viable alternative. Despite a higher false-positive rate (PPV 31.82% vs. 46.15%) and lower specificity, their simplicity, low infrastructure demands, and suitability for paramedical staff offer a cost-effective "screen-and-treat" approach with immediate results, reducing follow-up issues. VIA and VILI also aid in determining biopsy sites in areas without colposcopy services.

Noncytological screening methods could shift the approach to detecting cervical cancer precursors, raising awareness, and preparing the ground for HPV-based screening and vaccination programs. Our study highlights the benefits of using visual inspection-based screening, especially in low-resource and rural settings.

Recommendations

The advantages of affordability, straightforward implementation, and a point-of-care diagnosis and treatment protocol should serve as compelling reasons for developing countries like India to adopt visual inspection as a screening method for cervical cancer.

Patient Consent

Informed patient consent was obtained for this study.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. The resources used in the research were totally provided by the hospital and the Municipal Corporation of Delhi. Informed consent was obtained from all individual participants included in the study.

Funding

The study was entirely funded by Kasturba Hospital (Municipal Corporation of Delhi).

Conflict of Interest

None declared.

Acknowledgments

The authors would like to thank all the previous researchers, authors, and publishers of the articles whose work has been referenced and discussed in this research. We also wish to express our gratitude to all the participants of the study for their valuable contribution.

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