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Diagnostic accuracy of VIA and VILI for cervical pre-invasive lesions: A prospective cross-sectional study

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Abstract:
Cervical cancer screening using visual inspection with acetic acid (VIA) and Lugol's iodine (VILI) offers inexpensive and accessible methods for detecting cervical lesions. Therefore, it is of interest to compare the diagnostic accuracy of VIA and VILI using histopathology as the reference standard. A total of 94 women underwent Pap smear examination, VIA, VILI, and protocol-directed cervical biopsy. VIA showed a sensitivity of 96.2% and specificity of 45.6%, whereas VILI showed a sensitivity of 100% and specificity of 47.1%. Thus, both methods showed high sensitivity but limited specificity, emphasizing the need for confirmatory evaluation of positive screening results.

Keywords: Cervical cancer, cervical intraepithelial neoplasia, visual inspection with acetic acid, visual inspection with Lugol's iodine, cervical cancer screening, diagnostic accuracy

Background:
Cervical cancer remains a major public health challenge worldwide, with GLOBOCAN 2020 estimates reporting 604,127 new cases and 341,831 deaths globally. The majority of this burden is concentrated in low- and middle-income countries, where inadequate healthcare infrastructure, screening programs and follow-up systems contribute to poor outcomes. In India specifically, the disease poses a particularly heavy toll, with the country accounting for 21% of new cervical cancer cases in 2020 and an age-standardized incidence rate of 17.7 per 100,000 women. These disparities underscore the preventable nature of cervical cancer through early detection and treatment of pre-invasive lesions, particularly highlighted by the WHO's Global Cervical Cancer Elimination Initiative **[1]**. The conventional Pap smear, introduced in 1941, is based on the cytological and morphological assessment of exfoliated cervical cells to detect pre-invasive lesions of the cervix **[2]**. Despite its established value, the Pap smear exhibits several important limitations. Human papillomavirus DNA testing is a highly sensitive alternative screening strategy for detecting high-risk HPV, the causal agent in cervical cancer and is recommended in high-income countries for its cost-effectiveness and suitability across settings **[3, 4]**. Despite superior performance, implementation in low-resource settings faces barriers like high upfront costs, equipment needs and long processing times. Visual inspection with acetic acid is based on the principle that when dilute acetic acid is applied to cervical epithelium, it causes reversible coagulation or precipitation of cellular proteins, particularly in areas with increased cellular activity and DNA content. Areas with high protein content, such as dysplastic lesions and invasive cancer, undergo maximal coagulation which prevents light from passing through the epithelium, resulting in a dense white appearance known as the acetowhite reaction **[5]**. Despite these advantages, VIA exhibits significant limitations. The test has high inter-operator variability, making diagnostic accuracy highly dependent on the skill and experience of healthcare providers. Visual inspection with Lugol's iodine, also known as Schiller's test, is based on the principle that iodine is glycophilic and is selectively taken up by glycogen-containing epithelium **[6]**. When comparing VIA and VILI, studies have found that both tests are inexpensive, require minimal infrastructure, allow for immediate treatment and eliminate the need for laboratory facilities **[7]**. Therefore, it is of interest to evaluate and compare the diagnostic accuracy of visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) for detecting cervical pre-invasive lesions, using histopathology as the reference standard.

Materials and Methods:
This hospital-based prospective cross-sectional diagnostic accuracy study (observational analytical study) was conducted over a period of 18 months in the Department of Obstetrics and Gynecology, Rajendra Institute of Medical Sciences (RIMS), Ranchi, and Jharkhand, a tertiary care teaching hospital catering to both urban and rural populations. Women attending the Obstetrics and Gynecology outpatient department who fulfilled the eligibility criteria were consecutively enrolled after obtaining written informed consent. Women aged 25–70 years with abnormal Pap smear reports, high-risk HPV positivity, abnormal cervical appearance on per speculum examination, or presenting with recurrent and refractory vaginal discharge or postcoital bleeding were included. Women with a history of hysterectomy, cervical intraepithelial neoplasia, previous cervical surgery or treatment, allergy to acetic acid or iodine, severe physical or mental illness limiting participation, or pregnancy were excluded. Data were collected using a predesigned and pretested structured case record form containing socio-demographic details, menstrual and obstetric history, presenting complaints, clinical findings and investigation results. Following a per speculum examination, a conventional Pap smear was obtained from all participants. Subsequently, 5% acetic acid was applied to the cervix for Visual Inspection with Acetic Acid (VIA) and the presence of a distinct acetowhite area in the transformation zone after 1–2 minutes was considered a positive test. After washing the cervix with normal saline, Lugol's iodine was applied for Visual Inspection with Lugol's Iodine (VILI), where mustard-yellow areas indicating absent iodine uptake were considered positive. Cervical biopsy with endocervical curettage was performed in all women with a positive screening test and in 10% of screen-negative women. Histopathological examination served as the gold standard, with Cervical Intraepithelial Neoplasia (CIN I or higher) considered positive

for pre-invasive cervical lesions. Ethical approval was obtained from the Institutional Ethics Committee of RIMS, Ranchi and written informed consent was obtained from all participants. Confidentiality was maintained throughout the study and participants retained the right to withdraw at any stage without affecting their routine care. Data were entered into Microsoft Excel and analyzed using SPSS version 21. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized as mean ± standard deviation. The diagnostic performance of VIA and VILI was evaluated by calculating sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy using histopathology as the reference standard. Associations between screening test results and histopathological findings were assessed using the Chi-square test, with a p-value of <0.05 considered statistically significant and 95% confidence intervals calculated where appropriate. The ROC displays for the dichotomous VIA and VILI outcomes were reconstructed from their reported sensitivity and specificity; without participant-level scores or multiple thresholds, their AUC equals balanced accuracy and is descriptive rather than a conventional fitted ROC estimate.

Results:

A total of 94 women fulfilling the eligibility criteria were included in the study. The socio-demographic characteristics are presented in **Table 1**. The majority of participants belonged to the 35–44 years age group (26.6%), followed by the 55–64 years age group (23.4%). Most participants were married (41.5%), belonged to the middle socioeconomic class (26.6%) and had a parity of 6–9 (39.4%).

The clinical characteristics of the study participants are summarized in **Table 2**. Most women had regular menstrual cycles (52.1%) and menstrual flow lasting more than four days (62.8%). The majority reported monogamous sexual relationships (71.3%). Postmenopausal bleeding (60.6%) was the most common presenting complaint, followed by recurrent vaginal discharge (53.2%), intermenstrual bleeding (46.8%), postcoital bleeding (45.7%) and lower abdominal pain (43.6%), as illustrated in **Figure 1**. The distribution of screening test results and histopathological findings is shown in **Table 3**. Both VIA and VILI were positive in 66.0% of participants. On histopathological examination, 37.2% had normal findings and 34.0% had chronic cervicitis, while CIN II (12.8%) was the most common pre-invasive lesion. The overall distribution of histopathological diagnoses is depicted in **Figure 2**. The diagnostic performance of VIA and VILI using histopathology as the reference standard is presented in **Table 4**. VIA demonstrated a sensitivity of 96.2%, specificity of 45.6% and diagnostic accuracy of 59.6%, whereas VILI demonstrated 100% sensitivity, 47.1% specificity and 61.7% diagnostic accuracy. Receiver operating characteristic (ROC) curves for VIA and VILI are presented in **Figure 3** and **Figure 4**, respectively. Although VILI showed slightly better diagnostic performance than VIA, the difference between the two screening methods was not statistically significant (McNemar test, p = 0.909). The reported McNemar comparison also requires confirmation against a patient-level paired VIA–VILI contingency table.

Table 1: Socio-demographic and clinical characteristics of study participants (n = 94)

Variable	Category	n (%)
Age group (years)	25–34	19 (20.3)
	35–44	25 (26.6)
	45–54	19 (20.3)
	55–64	22 (23.4)
	65–70	9 (9.6)
Marital status	Married	39 (41.5)
	Unmarried	16 (17.1)
	Widowed	26 (27.6)
	Divorced	13 (13.8)
Education	Illiterate	15 (16.0)
	Primary	21 (22.3)
	Secondary	21 (22.3)
	Higher Secondary	19 (20.2)
	Graduate	18 (19.1)
Socioeconomic status	Lower	24 (25.5)
	Lower Middle	13 (13.8)
	Middle	25 (26.6)
	Upper Middle	13 (13.8)
	Upper	19 (20.2)
Parity	0–1	16 (17.0)
	2–5	34 (36.2)
	6–9	37 (39.4)
	≥10	7 (7.4)

Footnote: *Values are expressed as frequency (percentage).*

Table 2: Menstrual, reproductive and clinical characteristics of study participants (n = 94)

Variable	Category	n (%)
Age at menarche	≤12 years	40 (42.6)
	13–14 years	24 (25.5)
	≥15 years	30 (31.9)
Cycle regularity	Regular	49 (52.1)
	Irregular	45 (47.9)
Cycle duration	≤28 days	25 (26.6)
	>28 days	69 (73.4)
Duration of menstrual flow	≤4 days	35 (37.2)
	>4 days	59 (62.8)
Dysmenorrhea	Yes	47 (50.0)
	No	47 (50.0)
Sexual relationship	Monogamous	67 (71.3)
	Polygamous	11 (11.7)
	None	16 (17.0)
Duration of sexual life	<16 years	9 (9.6)
	≥16 years	85 (90.4)
Presenting symptoms*	Recurrent vaginal discharge	50 (53.2)
	Postcoital bleeding	43 (45.7)
	Intermenstrual bleeding	44 (46.8)
	Postmenopausal bleeding	57 (60.6)
	Lower abdominal pain	41 (43.6)

Footnote: **Multiple responses were allowed for presenting symptoms; therefore, percentages do not total 100%.*

Table 3: Distribution of screening test results and histopathological findings (n = 94)

Variable	Category	n (%)
Pap smear	NILM	12 (12.8)
	ASCUS	16 (17.0)
	LSIL	26 (27.7)
	HSIL	16 (17.0)
	Inflammatory	24 (25.5)
VIA	Positive	62 (66.0)
	Negative	32 (34.0)
VILI	Positive	62 (66.0)
	Negative	32 (34.0)
Histopathology	Normal	35 (37.2)

	Chronic cervicitis	32 (34.0)
	CIN I	6 (6.4)
	CIN II	12 (12.8)
	CIN III	4 (4.3)
	Carcinoma in situ	1 (1.1)
	Invasive carcinoma	4 (4.3)

**Abbreviations:* *NILM = Negative for Intraepithelial Lesion or Malignancy; ASCUS = Atypical Squamous Cells of Undetermined Significance; LSIL = Low-grade Squamous Intraepithelial Lesion; HSIL = High-grade Squamous Intraepithelial Lesion; VIA = Visual Inspection with Acetic Acid; VILI = Visual Inspection with Lugol's Iodine; CIN = Cervical Intraepithelial Neoplasia.*

Table 4: Diagnostic performance of VIA and VILI using histopathology as the reference standard (n = 94)

Parameter	VIA	VILI
True Positive (TP)	25	26
False Positive (FP)	37	36
True Negative (TN)	31	32
False Negative (FN)	1	0
Sensitivity (%)	96.2	100.0
Specificity (%)	45.6	47.1
Positive Predictive Value (%)	40.3	41.9
Negative Predictive Value (%)	96.9	100.0
Diagnostic Accuracy (%)	59.6	61.7
Positive Likelihood Ratio	1.77	1.89
Negative Likelihood Ratio	0.08	0.00*

*Histopathology (CIN I or higher) was considered the reference standard for disease positivity. *Negative likelihood ratio for VILI approached zero because no false-negative cases were observed. McNemar test comparing VIA and VILI: $\chi^2 = 0.0417$, $p = 0.909$, indicating no statistically significant difference in diagnostic performance.*

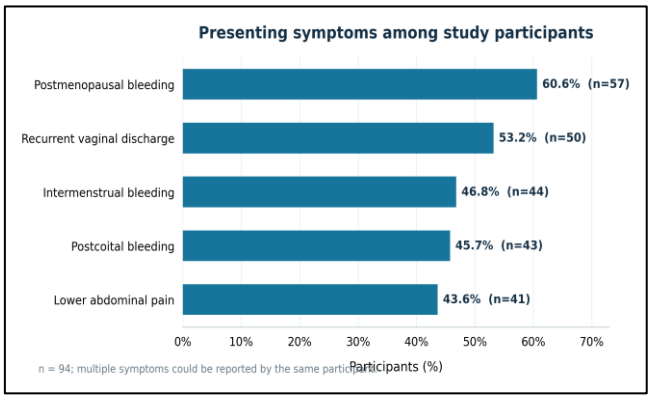


Figure 1: Distribution of study participants based on presenting symptoms

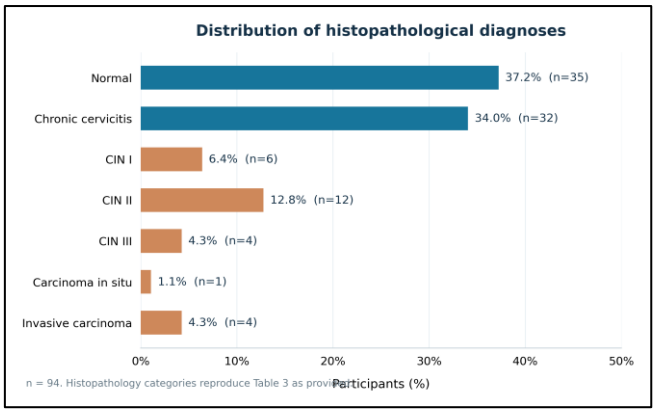


Figure 2: Distribution of study participants based on histopathology findings

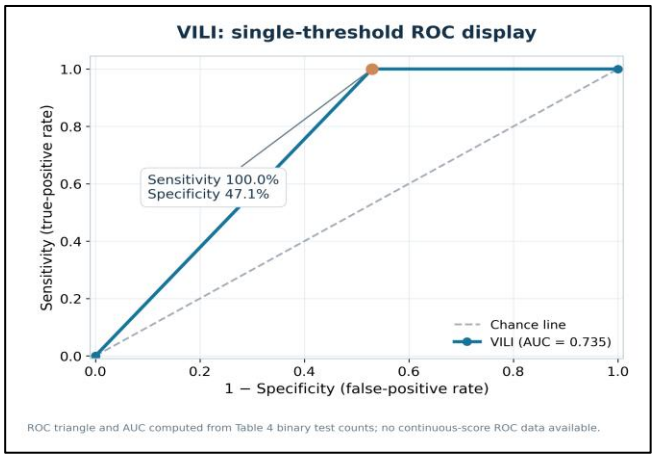


Figure 3: VILI single-threshold ROC (AUC = 0.735)

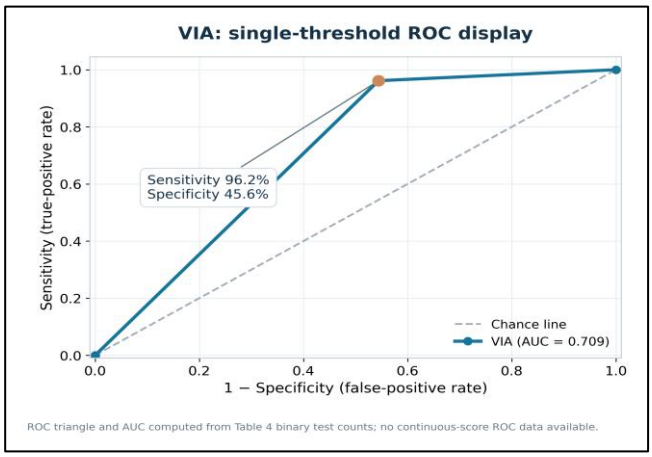


Figure 4: VIA single-threshold ROC (AUC = 0.709)

Discussion:

Cervical cancer presents a substantial global health challenge, with worldwide estimates in 2020 indicating 604,127 new cases and 341,831 deaths. A pronounced socioeconomic gradient exists, with incidence rates approximately three times higher and mortality rates nearly six times higher in countries with a low Human Development Index (HDI) compared with countries with a very high HDI. This disparity is further underscored by the fact that approximately 80% of cervical cancer cases occur in the developing world, where limited resources frequently constrain effective prevention, screening and early detection efforts **[8]**. In contrast, developed countries generally report lower incidence and mortality rates, largely because of greater access to organized cervical cancer screening programs, HPV vaccination and timely management of precancerous lesions **[9]**. Visual inspection with acetic acid (VIA) offers several advantages, particularly in low-resource settings. It is a simple and inexpensive screening method that can be performed by trained mid-level healthcare workers without the need for sophisticated laboratory infrastructure. The test requires

relatively short training periods and minimal equipment. Importantly, VIA provides immediate point-of-care results, thereby facilitating a "screen-and-treat" approach in which treatment or referral decisions can be made during the same visit. This approach is particularly valuable in settings where loss to follow-up is a major limitation of conventional multi-visit screening programs **[10]**. Despite these advantages, VIA have important limitations. Its diagnostic performance is influenced considerably by inter-observer variability and depends on the training and experience of the examiner. Interpretation of acetowhite changes is subjective and may also be affected by the availability of supplies, adherence to procedural guidelines, cervical anatomy, inflammation and menopausal status. Although VIA demonstrates reasonable accuracy for detecting high-grade cervical precursor lesions, its specificity may be suboptimal, particularly among older women and in women with endocervical or inflammatory lesions, resulting in an increased frequency of false-positive findings **[11]**. When compared with VIA, visual inspection with Lugol's iodine (VILI) has demonstrated relatively high sensitivity in several studies. Multicenter investigations have reported VILI sensitivity ranging from approximately 76% to 97%, whereas VIA sensitivity has generally ranged from 67% to 79% **[12]**. The specificity of the two tests, however, shows considerable overlap across studies. The iodine-based staining used in VILI generally provides greater visual contrast and clearer delineation of abnormal epithelial areas than the acetowhite reaction produced during VIA. This may facilitate recognition of subtle precancerous lesions that are less readily identified using VIA alone. Both VIA and VILI remain inexpensive screening approaches that require minimal infrastructure, provide immediate results and can be integrated with same-visit management strategies without dependence on laboratory facilities **[13]**. In the present study (n = 94), VIA showed an apparent positivity rate of 66.0%, whereas the prevalence of histopathologically confirmed disease was 27.7%, demonstrating a considerable burden of false-positive VIA findings. VIA exhibited very high sensitivity of 96.2% (95% CI: 80.4–99.9) and an excellent negative predictive value (NPV) of 96.9% (95% CI: 83.8–99.9). Thus, a negative VIA result was highly reassuring for excluding histopathology-positive cervical disease. In contrast, VIA demonstrated relatively low specificity of 45.6% (95% CI: 33.5–58.2), with a false-positive proportion of 54.4% and a positive predictive value (PPV) of 40.3%. Consequently, the overall diagnostic accuracy of VIA was 59.6%. The likelihood ratios showed a similar pattern: an LR+ of 1.77 indicated limited ability to confirm disease, whereas an LR- of 0.08 indicated strong potential for excluding disease when the VIA result was negative. These findings are broadly consistent with the recognized clinical behavior of VIA, which generally demonstrates high sensitivity and NPV but variable specificity depending on the characteristics of the population, examiner experience and the positivity threshold applied **[10–12]**. In an Indian hospital-based study, Ghosh *et al.* reported VIA sensitivity of 89.5% and specificity of 91.2%, suggesting good diagnostic performance when visual criteria were applied within a structured clinical and verification setting **[14]**. The sensitivity observed in the present study was even higher at 96.2%; however, specificity was considerably lower at 45.6%. The relatively low specificity in the present investigation may be attributable to a spectrum and clinical-context effect. The study population was predominantly symptomatic and had a substantial burden of inflammatory cervical pathology. Such a clinically enriched population may demonstrate acetowhite reactions unrelated to cervical intraepithelial neoplasia (CIN), including changes associated with chronic cervicitis, cervical ectopy, immature squamous metaplasia and reparative epithelium. These conditions can increase false-positive classifications and consequently reduce specificity. This explanation is supported by the histopathological distribution in the current study, in which chronic cervicitis constituted approximately 34% of cases. Published estimates of VIA specificity vary considerably according to study population, lesion prevalence, verification methods, examiner expertise and the criteria used to define a positive test. In the present study, the relatively low specificity may therefore reflect the high prevalence of benign inflammatory and reparative changes together with the application of a sensitive positivity threshold in a symptomatic hospital-based population. These factors may increase false-positive findings while maintaining high sensitivity **[10–12, 15]**. VILI demonstrated an apparent positivity rate of 66.0%, similar to VIA. However, VILI achieved an observed sensitivity of 100% (95% CI: 86.8–100) and an NPV of 100% (95% CI: 89.1–100). Therefore, none of the histopathology-positive cases were missed by VILI in the present dataset. Despite this excellent sensitivity, specificity remained relatively low at 47.1% (95% CI: 35.0–59.6), with a false-positive proportion of 52.9%. The corresponding overall diagnostic accuracy was 61.7%, while the PPV was 41.9%. The likelihood ratios demonstrated a similar diagnostic pattern. An LR+ of 1.89 indicated limited confirmatory value for a positive VILI result, whereas the LR− approached zero because no false-negative cases were observed. The combination of very high sensitivity and NPV with comparatively low specificity is biologically plausible for VILI. Lugol's iodine identifies glycogen-containing mature squamous epithelium, whereas abnormal or immature epithelium remains iodine-negative. However, iodine non-uptake is not specific to CIN and may also occur in areas affected by inflammation, immature metaplasia, epithelial atrophy and cervical ectopy **[12, 13]**. The high prevalence of chronic cervicitis in the present population may therefore have contributed substantially to the number of false-positive VILI findings. The observed sensitivity of VILI in the present study is comparable to that reported by Ghosh *et al.*, who found a sensitivity of 100% with a specificity of 93.35% **[14]**. The principal difference between their findings and those of the present study relates to specificity, which was only 47.1% in the current population. Differences in case mix, disease spectrum, examiner interpretation, verification procedures and thresholds for defining iodine-negative areas as abnormal may explain this discrepancy. A more liberal definition of VILI positivity, including patchy or relatively faint areas of iodine non-uptake,

would be expected to increase sensitivity but decrease specificity. Other investigations have demonstrated that the relative performance of VIA and VILI is not uniform across different clinical populations. Some studies have reported very high specificity but lower sensitivity for VILI, whereas others have observed higher sensitivity for VILI than VIA **[15]**. Such variation may arise from differences in interpretation thresholds, lesion spectrum, examiner training, prevalence of inflammation, age distribution of the population and histopathological verification strategies. Therefore, VILI may function as either a highly sensitive or a highly specific screening test depending on how strictly iodine-negative findings are interpreted and on the characteristics of the population being screened. When VIA and VILI were directly compared in the present study, VILI demonstrated numerically better performance across most diagnostic parameters. Sensitivity was 100% for VILI compared with 96.2% for VIA, specificity was 47.1% versus 45.6%, diagnostic accuracy was 61.7% versus 59.6%, PPV was 41.9% versus 40.3% and NPV was 100% versus 96.9%, respectively. Nevertheless, these differences were small. The McNemar test showed no statistically significant difference between the paired performance of VIA and VILI (χ^2 = 0.0417, p = 0.909). The tendency toward slightly higher sensitivity with VILI is consistent with findings reported in several comparative investigations **[12, 14 and 15]**. However, published evidence also demonstrates considerable heterogeneity in the relative diagnostic performance of VIA and VILI. Taken together, these observations indicate that the direction and magnitude of differences between the two visual screening techniques are not universal and may vary according to examiner training, diagnostic threshold and prevalence of inflammatory cervical conditions, study setting and characteristics of the screened population **[12–15]**. The present findings demonstrate that both VIA and VILI can function as highly sensitive screening or triage methods in a symptomatic hospital-based population, with VILI demonstrating 100% observed sensitivity in the current sample. However, the relatively low specificity and modest PPV of both techniques indicate that a positive visual screening result should be interpreted as an indication for further diagnostic assessment rather than as definitive evidence of cervical precancer or cancer. The absence of a statistically significant difference between VIA and VILI (McNemar p = 0.909) suggests comparable overall performance in the present cohort. Accordingly, the choice between the two approaches may depend on local feasibility, availability of materials, examiner expertise, institutional protocols and screening-program logistics **[10–15]**. Histopathological confirmation remains important for appropriately selected screen-positive women, particularly in symptomatic populations in which inflammatory cervical abnormalities may contribute to false-positive visual findings. The present study has certain limitations that should be considered when interpreting its findings. First, because this was a hospital-based investigation conducted predominantly among symptomatic women attending a tertiary care center, the findings may not be directly generalizable to asymptomatic women or community-based cervical cancer screening populations. Second, the relatively small sample size of 94 participants may have limited the precision of diagnostic accuracy estimates, particularly the confidence intervals for sensitivity and specificity. Third, the high prevalence of chronic cervicitis may have contributed to the relatively low specificity of both VIA and VILI because inflammatory changes can produce visual appearances that resemble cervical precancerous lesions. Fourth, histopathological confirmation was performed in all screen-positive women but in only 10% of screen-negative women, potentially introducing partial verification bias. Finally, inter-observer variability in the interpretation of VIA and VILI was not formally assessed; consequently, the effect of examiner training and experience on diagnostic performance could not be quantified. Yadav *et al.* **[16]** reported sensitivity of 84.21% for VIA and 73.68% for VILI, with respective specificities of 80.0% and 60.0% in a hospital-based Indian study; their pattern differs from the present dataset and emphasizes variation with case selection, test criteria and reference verification. In a meta-analysis of 21 studies, Bharti *et al.* **[17]** estimated pooled VIA sensitivity of 80% (95% CI: 71–87%) and specificity of 81% (95% CI: 70–88%), contrasting with the exceptionally high sensitivity and low specificity recorded here. Swathi *et al.* **[18]** evaluated 100 women and reported screening positivity of 12% by VIA and 15% by VILI, with reported AUC values of 0.78 and 0.85, respectively.

Conclusion:

VIA and VILI are effective, low-cost and feasible screening tools with high sensitivity for early detection of cervical pre-invasive lesions, particularly in resource-limited settings. Nevertheless, due to their moderate specificity, positive cases should undergo confirmatory histopathological evaluation before definitive treatment planning. The integration of visual screening methods into routine gynecological practice may contribute substantially to the prevention of invasive cervical cancer.

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