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Comparing Results of VIA Testing for Cervical Cancer Screening with HPV DNA Testing at a Tertiary Care Centre

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Abstract

Background: Cervical cancer ranks fourth among the most commonly diagnosed cancers as well as the fourth leading cause of cancer mortality in women globally. The major risk factor for cervical cancer is persistent infection with high risk (HR) human papilloma virus (HPV) and 83.2% of invasive cervical cancers are attributed to HPVs 16 or 18[1]. In 2018, World Health Organization has called for a global action towards elimination of cervical cancer (a threshold of 4 per 100,000 women-year) and set 90-70-90 targets to be achieved by 2030[2]. The challenges and difficulties in implementing cytology screening in Low resource countries have stimulated the search for alternative methods of screening such as HPV Testing, visual inspection with acetic acid (VIA) or with Lugol's iodine - which provide result in less than 5 minutes, eliminate loss to follow up and give treatment in the same visit.

Methods: A hospital based cross sectional study was conducted in the Gynecology OPD of PBM Hospital, Bikaner. 118 women aged 19-60 years presenting with complaints such as intermenstrual bleeding, postmenopausal bleeding, increased or foul-smelling vaginal discharge etc were included. A questionnaire was administered containing general information, clinical findings at pelvic evaluation. They then underwent Visual Testing with Acetic Acid (VIA), HPV testing and histo-pathology. Appropriate statistical tests were used to compare the outcome between the sub-groups.

Results: On comparing VIA with HPV-DNA the sensitivity was 62.5%, specificity was 83.64%, PPV was 21.7% and NPV was 96.8%. The p-value was 0.001. VIA identified over half of HPV-DNA positive women and was highly reliable in ruling out HPV infection. Compared with Histopathology, its sensitivity was 100%, specificity was 82.61%, PPV was 13% and NPV was

100%. The p-value was <0.001. VIA detected all biopsy-confirmed disease cases, making it an excellent rule-out tool, despite many false positives.

Conclusions: VIA testing reliably rules out HPV infection, reflecting its utility as a screening test in early detection of high-risk cases in low and middle income countries.

Keywords: Cervix, Cervical Cancer Screening, VIA Testing, HPV DNA Testing.

Introduction

Cervical cancer ranks fourth among the most commonly diagnosed cancers as well as the fourth leading cause of cancer mortality in women globally[3]. Infection with HPV remains the major risk factor. Besides HPV, other factors include early age at first intercourse, multiple sexual partners, smoking, HIV infection, and lack of access to screening and vaccination programs.

In developing countries like India, down staging is employed. It is defined as a process of screening for cancer using clinical approaches for early detection. This is distinct from screening test and results in detection of the disease at a less advanced stage in the absence of screening. Paramedical staff is trained for minimum period to identify any abnormality including suspicious cervix and refer the cases early to centres where facilities exist for treatment of premalignant and malignant lesions, including educating the women regarding risk factors, symptoms of the disease and prophylaxis. A 25% reduction in cervical cancer incidence and a 35% reduction in cervical cancer mortality were found following a single round of VIA screening provided by trained nurses in a randomized trial in India[4]. The greatest reduction in incidence and mortality rates were observed for the 30- to 39-year age group which is explained by the fact that the transformation zone, where cervical neoplasia occurs, is fully exposed on the ectocervix in young women, enabling VIA to detect the abnormalities[5].

HPVs are small, non-enveloped viruses with an 8-kb circular double-stranded DNA contained in a 55 nm icosahedral capsid. The viral genome holds the long control region (LCR) that regulates genome replication and transcription of the early (E1-E7) and the late-expressed genes L1 and L2[6.] Synthesized E1 and E2 interact with the origin of replication site in the LCR. As the epithelium differentiates, the expression of the early viral genes, including E5 and E4, augments in the middle and upper layers, and genome amplification increases[7]. E6 can stimulate the transcriptional factor OCT-4 expression, which binds to the p53 promoter recruiting the NCOR1 co-repressor[8]. The E7 oncoprotein promotes cell cycle progression by sequestering and degrading the tumor suppressor protein pRB via polyubiquitination by cullin 2 (CUL2) (52) [9,10].

External cofactors are also crucial for HPV-induced carcinogenesis, as is the case of the microbiome. Alterations in the cervicovaginal microbiome occur during the progression of HPV-associated lesions to CC, where an increase in resident bacteria diversity occurs along with a reduction of the resident Lactobacillus spp. Dysbiosis in the cervical microbiome influences viral persistence and is a carcinogenic co-factor[11,12].

Hence cost-effective strategies are needed for screening and early diagnosis. The challenges and difficulties in implementing cytology screening in Low resource countries have stimulated the search for alternative methods of screening such as HPV Testing, visual inspection with acetic acid (VIA) or with Lugol's iodine - which provide result in less than 5 minutes, eliminate loss to follow up and give treatment in the same visit [13,14]. We therefore conducted this study to evaluate the efficacy of visual inspection using acetic acid (VIA),

HPV testing and PAP Smear by comparing the sensitivity, specificity, positive predictive value and negative predictive value.

Materials and Methods

A hospital based prospective study was conducted in the Gynecology OPD of PBM Hospital, Bikaner from June 2024 to May 2025. A sample size of 118 females was taken at 95% confidence level and 5% absolute error. We included non-pregnant women aged 19-60 years, willing to participate, presenting with complaints such as intermenstrual bleeding, postmenopausal bleeding, increased or foul-smelling vaginal discharge; persistent pain in the back/legs/pelvis; weight loss etc.

Our main objective was to study the efficacy of VIA testing and HPV testing by comparing the sensitivity, specificity, positive predictive value and negative predictive value. Based on the eligibility criteria, the participants were screened and selected from the OPD after a written and informed consent. Selected participants were interviewed using a semi-structured questionnaire with general information on each woman, clinical findings at pelvic evaluation, results of the VIA Testing and HPV testing.

Patients were placed in lithotomy position. The procedure was carried out with the assistance of a trained nurse. A speculum examination was then started using a Sim's speculum/ Cusco's self-retaining speculum, without any lubricant, with a direct visual evaluation of the cervix with the aid of a halogen focus lamp, to identify cervicitis, leukorrhea, polyps, ulcers, etc. After collection of cervical cells, all the women then underwent visual inspection with acetic acid. The vagina and cervix were cleaned with Normal Saline. 5% acetic acid was prepared by adding 5 ml of glacial acetic acid into 95 ml of distilled water and mixing thoroughly. As the dysplastic cells have more chromatin content, the coagulation is intense and cells turn white after application of acetic acid. HPV DNA Testing was done using a vaginal and a cervical sample. Sample was collected using disposable swab and then inserted in the liquid media provided and stored at -20 degree Celsius till processing. We used Hi-PCR® Human Papilloma Virus Genotyping[16,18] multiplex probe PCR Kit for HPV DNA detection.

All VIA positive cases and HPV DNA positive were followed by cervical biopsy for confirmation. The data was entered and analysed systematically. Appropriate statistical tests were used to compare the outcome between two sub-groups, considering cervical biopsy as the gold std. Quality assurance was ensured.

Results & Discussion

The present prospective study was conducted among 118 women presenting with high risk factors for development of cervical cancer at the Out Patient Department of Obstetrics and Gynecology in PBM Hospital, Bikaner. As per the inclusion criteria, the participants were evaluated based on their clinical profile, risk factors. The diagnostic performance of different cervical cancer screening tools - VIA and HPV DNA were compared using histopathology as the reference standard. The analysis covered demographic, reproductive, clinical, and diagnostic variables.

The participants were aged from 19 to 60 years, with a mean age of 37.09 years and a standard deviation of 10.63 years. The largest proportion (35%) of cases belonged to the 30–39-year age group. Overall, 99% of the cases were below 50 years, indicating that the condition predominantly affected women in their reproductive and peri-menopausal years. This distribution indicates that a substantial proportion of cervical abnormalities and potential early lesions are being detected in younger women, particularly in the 30–39 age group, which accounts for more than one-third of

the sample. This trend aligns with regional epidemiological studies from across India. H. Kumar (2025)[15] found that in a tertiary hospital in Southern India, 41% of patients were in the 30–49 years age group, reinforcing the early age at presentation. The study attributed early onset to early marriage, early sexual debut, and high parity in rural populations. Ray et al. (2025)[16] conducted a molecular epidemiology study in North-East India and reported high HPV prevalence in the 31–45-year group, which correlates with the observed high incidence of cervical cancer precursors in that demographic. The Lancet Southeast Asia projections (2025)[17] observed that India's cervical cancer incidence is shifting slightly to younger age brackets due to demographic changes and better awareness in urban regions, though rural and peri-urban areas still show late-stage presentation in older women.

Parity analysis showed that the majority of women were multiparous. The physiological rationale included prolonged exposure of the cervical epithelium to hormonal fluctuations and repeated cervical dilation during childbirth. According to ICMR-NCDIR (2021) data, multiparity (>3 children) was found in over 60% of cervical cancer patients in rural and semi-urban areas of India, suggesting sociocultural factors like early marriage and preference for larger families play a role. A variety of symptoms were reported, often in combination. The most prevalent complaint was white vaginal discharge, reported by 86.44% of women, followed by lower abdominal pain (79.66%), and backache (71.19%). Bahadur et al. (2024)[18] found white discharge (81%), abdominal pain (69%), and backache (59%) as top symptoms in patients undergoing cervical cancer screening. Kumar et al. (2022)[19] noted that contact bleeding and postmenopausal bleeding, though less common, were highly predictive of cervical lesions. Sharma et al. (2020)[20] reported that dyspareunia and dysuria were common in women with chronic cervicitis and early neoplastic changes, especially among rural women with poor hygiene. Persistent or combined symptoms, especially in women over 30, should prompt cytology, HPV testing, or biopsy, even in the absence of visible lesions. This pattern is consistent with other Indian studies and supports the need for early evaluation in resource-limited settings where routine screening may be lacking.

VIA Screening was found to be positive in 23 women (19.49%) and negative in 95 (80.5%). Out of 118 patients, 8 tested positive (6.8%) for high-risk HPV. Biopsies were then conducted for women reporting any of the 2 screening tests as positive or a strong suspicion on examination.

Table 1: Comparison of VIA results with HPV DNA status

	HPV DNA positive (8)	HPV DNA negative (110)	p-value	Sensitivity; Specificity	PPV; NPV
VIA positive (23)	5	18	0.001	62.5%; 83.64%	21.7%; 96.8%
VIA negative (95)	3	92			

On comparing VIA results with HPV DNA Testing results, the Sensitivity was found to be 62.5% and Specificity was 83.64% i.e. VIA correctly identified 92 of the 110 women who were HPV DNA negative. Positive Predictive Value (PPV) was found to be 21.7%. This shows a high rate of false positives, meaning many women would be unnecessarily referred for follow-up while the Negative Predictive Value (NPV) was 96.8% i.e. among those who tested negative with VIA, over 98% were truly HPV negative. The p-value was found to

be 0.001. There is a statistically significant association between VIA and HPV DNA results, suggesting that VIA has some predictive utility — though its performance is weaker compared to HPV DNA.

Table 2: Comparison of VIA results with histopathology

	Biopsy positive (3)	Biopsy negative (115)	p-value	Sensitivity; Specificity	PPV; NPV
VIA positive (23)	3	20	<0.001	100%; 82.61%	13%; 100%
VIA negative (95)	0	95			

On comparing VIA results with histopathology results, the Sensitivity was found to be 100% and Specificity was 82.61% - VIA correctly identified 82.61% of true negative cases (95/115 total biopsy-negative cases). The Positive Predictive Value (PPV) was 13% while the Negative Predictive Value (NPV) was 100% i.e. all 95 VIA negative results were truly negative, showing perfect reliability of a negative VIA test.

In a similar study by Sankaranarayanan et al.[21], comparing VIA to HPV testing sensitivity of VIA was ~60%-70%, NPV of VIA was >95%. Bhatla et al[12] in another study reported that VIA missed 30–35% of hrHPV infections, especially in asymptomatic or younger women. Joshi et al.[22] in a genotyping study, found out that 60% of hrHPV-positive women were VIA-negative, reinforcing that VIA cannot replace HPV testing in high-resource settings. Chaudhary et al.[23] reported VIA sensitivity of 58.6% and NPV of 96.7% against HPV DNA testing among women aged 30–50 in urban slums. The findings affirm that VIA is a valuable, low-cost tool for cervical screening, which can be used as a triage or initial screening tool especially in rural or low-resource settings. Its high NPV and moderate specificity make it effective for excluding disease.

Gupta et al[24]. in a similar study found sensitivity of VIA in comparison with histopathology to be 100%, specificity was 79.6%, PPV was 23%, and NPV was 100%. Swaminathan et al.[25] in another study found that VIA had a NPV of 99.1%; 100% sensitivity and a PPV of 17.91% and moderate specificity suggesting that VIA-positive women should be triaged with Pap smear, colposcopy, or biopsy to avoid overtreatment.

VIA is cheaper and easier to implement in resource-limited settings, often enabling same-day screening and treatment. HPV DNA tests require laboratory infrastructure and may delay results, potentially affecting follow-up in Low and Middle Income Countries.

Conclusions

Cancer cervix has a long preinvasive stage of 10-15 years. Screening programme has proved effective in reducing incidence of invasive cancer by 80% and mortality by 60%. Cervical cancer screening is crucial for early detection and prevention. The primary methods used are Pap Test (Pap Smear), HPV Test, Co-testing (HPV and Pap Testing) and Visual Inspection with Acetic Acid (VIA).

These findings validate that VIA is a simple, highly sensitive, low-cost test ideal for primary screening, especially due to its perfect NPV. HPV DNA testing is the most accurate screening tool, with perfect sensitivity and NPV. False positives were minimal. HPV DNA testing as the most sensitive and reliable tool for early detection of cervical neoplasia. The high NPV (100%) ensures that a negative test result provides strong reassurance against cervical disease.

With evidence-backed strategies and scalable implementation, cervical cancer elimination is within reach. The WHO has called for 90% of girls vaccinated,

70% of women screened, and 90% of those treated as part of its 2030 elimination initiative. This study supports that vision by demonstrating the power of simple, combined screening methods to detect, prevent, and ultimately eliminate cervical cancer in India.

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Abbreviations

High risk (HR)

Human papilloma virus (HPV)

Visual Testing with Acetic Acid (VIA)

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