

# Diagnostic utility of pap smear and visual inspection of acetic acid for the detection of various cervical lesions



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Submission: 14-04-2022

Revision: 27-07-2022

Publication: 01-09-2022

## ABSTRACT

**Background:** Cervical cancer is one of the leading causes of death among women facing gynecological problems. It requires early detection by various screening methods. **Aims and Objectives:** The current research aimed to assess the diagnostic utility of pap smear cytology and visual inspection of acetic acid (VIA) as a screening test for various cervical lesions. **Materials and Methods:** The current prospective study was conducted on 210 non-pregnant women. Cervical pap smears were taken and reported according to the revised Bethesda system, 2014. VIA examination was also done, and any dense opaque aceto-white lesion observation near the squamo-columnar junction was reported as VIA positive. The findings of pap smear cytology and VIA were correlated with findings of histopathology. **Results:** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of pap smear cytology for the diagnosis of cervical lesions were found to be 90.16%, 85.22%, 89.43%, 86.2%, and 88.09%, respectively; while sensitivity, specificity, PPV, NPV, and diagnostic accuracy of VIA for the diagnosis of cervical lesions were found to be 88.37%, 55.55%, 76%, 75%, and 75.71%, respectively. Our study showed a substantial degree of agreement between pap smear cytology and histopathology for the diagnosis of cervical lesions with Cohen's kappa value being 0.755. **Conclusion:** The current study revealed that pap smear and VIA have high sensitivity with good diagnostic accuracy for the detection of neoplastic cervical lesions, which indicate that they can be used as an efficient screening tool for the detection of cervical lesions in their earlier stages.

**Keywords:** Cervical lesions; Cytology; Pap smear; Screening test; VIA

### Access this article online

**Website:**

<http://nepjol.info/index.php/AJMS>

**DOI:** 10.3126/ajms.v13i9.44448

**E-ISSN:** 2091-0576

**P-ISSN:** 2467-9100

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## INTRODUCTION

In India, carcinoma of the breast and cervix is a major health problem among women of reproductive and post-reproductive ages.<sup>1</sup> Both cancers not only create a financial burden on the nation but also reduce the quality of the life of the patients. Cervical cancer is the 4<sup>th</sup> leading cause of cancer-associated death in females worldwide.<sup>2</sup> Various screening tests have been developed aiming to detect these cancers at an early stage. Cervical screening programs implementing the use of pap smear cytology have shown a reduction in the incidence of cervical carcinoma greatly.<sup>3</sup> Visual inspection of acetic acid (VIA) is an attractive alternative to pap smear cytology for its ease

to usage, lower cost than pap smear, and fewer physician visits required.<sup>4</sup>

### Aims and objectives

The present study aimed to assess the utility of pap smear cytology and VIA for the detection of non-malignant, pre-malignant and malignant lesions of cervix.

## MATERIALS AND METHODS

The current prospective study was conducted at a tertiary care center of Madhya Pradesh, India. After taking permission from the Institutional Ethics Committee, 210

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non-pregnant women in the age group of 21–65 years, attending the Gynecology outpatient department for various gynecological problem were selected, who underwent both pap smear and cervical biopsy or hysterectomy. Pregnant women and those women who have been treated earlier for cervical carcinoma or are presently on cancer treatment or had undergone any cervical surgery or hysterectomy were not part of the present study. Detailed obstetrical history, history of contraception, menstrual history, history of white vaginal discharge, pelvic pain, and post-coital bleeding were noted after taking consent. Patients were explained the pap smear and VIA tests in detail.

#### Procedure and interpretations of pap smear

An examination of the vagina and cervix was done by a speculum, and a circumferential sample was obtained by scraping the squamo-columnar junction by the Ayre's spatula. The sample was shifted on the glass slide and smear was prepared. All smears were fixed with 95% ethyl alcohol and stained by Papanicolaou stain. All the stained slides were examined under the microscope. The test result of pap was divided into two categories; pap positive and pap negative. The cytological diagnosis of negative for intra-epithelial lesion or malignancy (NILM) was considered as pap negative, whereas the cytological diagnosis of atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (SCC) were considered as pap positive.<sup>5</sup>

#### Procedure and interpretations of VIA

About 5% acetic acid solution was applied on the cervix using a cotton swab. After waiting for a while, a visual inspection of the cervix was done by the gynecologist. Observation of well-defined dense opaque aceto-white lesion close to the squamo-columnar junction or aceto-white area touching the transformation zone was reported as VIA positive. On the other hand, if no aceto-white lesion or area was observed, it was reported as VIA negative.

#### Histopathological examination (HPE)

Histopathological processing was done for all biopsies and stained with Hematoxylin and Eosin stain. All stained slides were examined under a light microscope, and histopathological findings were noted. Histopathological findings were taken as a gold standard to compare results of VIA and pap smear.

#### Correlations between HPE and pap smear or VIA findings

The cases which were diagnosed as neoplastic cervical lesions by both histopathology and pap smear or VIA findings were considered as true positive (TP), whereas

the cases which were diagnosed as neoplastic cervical lesions by pap smear or VIA examination but diagnosed as non-neoplastic cervical lesions by histopathology were considered as false positive (FP). The cases which were diagnosed as non-neoplastic cervical lesions by both histopathology and pap smear or VIA findings were considered as true negative (TN), whereas the cases which were diagnosed as non-neoplastic cervical lesions by pap smear or VIA examination but diagnosed as neoplastic cervical lesions by histopathology were considered as false negative (FN).

#### Statistical analysis

Analysis of data was done using Statistical Package for the Social Sciences ver. 22 (Chicago), IL. Categorical data were expressed as frequency counts (percentages). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of pap smear and VIA results for the diagnosis of cervical lesions were calculated using a standard statistical formula. An agreement between two different (histological and pap smear or VIA) methods was interpreted by evaluating Cohen's kappa ( $\kappa$ ) value.

## RESULTS

In the present study, 210 women were screened for pre-cancerous and cancerous cervical lesions using pap smear cytology and VIA. Patients were between the ages of 23–58 years, with the maximum incidence in the 4<sup>th</sup> decade.

#### Pap smear findings

In cytological diagnosis, 41.42% (87/210) cases of cervical lesions were diagnosed as NILM, whereas 11.42% (24/210) cases were diagnosed as ASC-US. About 32.85% (69/210) cases were diagnosed as LSIL, whereas 12.85% (27/210) cases were diagnosed as HSIL. Only 1.42% (3/210) cases were diagnosed as SCC (Table 1).

#### Histopathology findings

On HPE, 41.90% (88/210) cases were diagnosed as chronic cervicitis. CIN-I, CIN-II, and CIN-III were diagnosed, respectively, in 40.47% (85/210), 13.33% (28/210), and 2.85% (6/210) cases. About 1.42% (3/210) cases were diagnosed as SCC on HPE (Table 1).

#### Comparison of pap smear and HPE findings

Out of 87 cytologically diagnosed NILM cases, 75 cases were diagnosed as chronic cervicitis on HPE (Table 1). NILM and chronic cervicitis are non-neoplastic cervical lesions.<sup>5</sup> Therefore, NILM findings of pap smear and Chronic cervicitis findings of HPE correspond to each other. So these, 75 cases were considered as TN (Tables 1 and 2). Pap smear misdiagnosed 12 NILM cases,

**Table 1: Comparison of cytological findings of cervical lesions with histopathological findings (n=210)**

Cytological diagnosis (Pap smear results)	Histopathological diagnosis					Total
	Chronic cervicitis	CIN I*	CIN II*	CIN III*	SCC†	
NILM <sup>§</sup>	75	10	02	-	-	87 (41.42%)
ASC-US <sup>§§</sup>	07	15	02	-	-	24 (11.42%)
LSIL <sup>‡</sup>	06	60	03	-	-	69 (32.85%)
HSIL <sup>‡‡</sup>	-	-	21	06	-	27 (12.85%)
SCC <sup>†</sup>	-	-	-	-	03	03 (1.42%)
Total	88 (41.90%)	85 (40.47%)	28 (13.33%)	06 (2.85%)	03 (1.42%)	210 (100%)

<sup>§</sup>Negative for intra-epithelial lesion or malignancy, <sup>§§</sup>Atypical squamous cells of undetermined significance, <sup>‡</sup>Low grade squamous intra-epithelial lesions, <sup>‡‡</sup>High grade squamous intra-epithelial lesions, <sup>\*</sup>Cervical intraepithelial neoplasia, <sup>†</sup>Squamous cell carcinoma). Yellow and orange color boxes indicate true negative (TN) and true positive (TP) cases respectively. Tan and purple color boxes indicate false negative (FN) and false positive (FP) cases respectively.

**Table 2: Comparison of pap smear findings with histopathological findings in diagnosis of cervical lesions (n=210)**

Cytological diagnosis (Pap smear findings)	Histopathological diagnosis	Histopathological diagnosis		Total
		Neoplastic (+)	Non neoplastic (-)	
Neoplastic (+)		110 (TP)	13 (FP)	123
Non-neoplastic (-)		12 (FN)	75 (TN)	87
Total		122	88	210
Interpretation of the pap smear screening test		Sensitivity=TP/(TP+FN) = 90.16% Specificity=TN/(TN+FP) = 85.22% Positive Predictive Value (PPV) =TP/(TP+FP) =89.43% Negative Predictive Value (NPV) =TN/(TN+FN) =86.2% Diagnostic Accuracy= (TP+TN)/(TP+FN+FP+TN) = 88.09% Cohen's Kappa value ( $\kappa$ ) = 0.755		

The orange color box indicates true positive (TP) cases. Purple color box indicates false positive (FP) cases. Tan color box indicates false negative (FN) cases. Yellow color box indicates true negative (TN) cases.

which were correctly diagnosed as CIN-I (10 cases) and CIN-II (2 cases) by HPE. These 12 cases were considered as FN (Tables 1 and 2).

Among the 24 cytologically diagnosed ASC-US cases, 15 cases were diagnosed as CIN-I and 2 cases were diagnosed as CIN-II on HPE. Out of the 69 cytologically diagnosed LSIL cases, 60 cases were diagnosed as CIN-I, and three cases were diagnosed as CIN-II on HPE. Among 27 cytologically diagnosed HSIL cases, 21 cases were diagnosed as CIN-II, and six cases were diagnosed as CIN-III on HPE. Cytological diagnosis of all the three cases of SCC was consistent with HPE (Table 1). ASC-US, LSIL, HSIL, and SCC, all are cervical neoplastic lesions.<sup>5</sup> Hence, a total of 110 cases were diagnosed as neoplastic cervical lesions on both HPE and cytological examination. These 110 cases were considered as TP (Tables 1 and 2). Pap smear misdiagnosed 7 ASC-US and 6 LSIL cases, which were correctly diagnosed as chronic cervicitis by HPE. Hence, these 13 cases were considered as FP (Tables 1 and 2).

In our study, diagnostic sensitivity and specificity of pap smear in detecting neoplastic and non-neoplastic cervical lesions were found to be 90.16% and 85.22%, respectively. PPV was found to be 89.43% and NPV was found to be 86.2%. The overall diagnostic accuracy of pap smear in

detecting neoplastic cervical lesions was found 88.09%. Cohen's kappa ( $\kappa$ ) value was found to be 0.755, which shows that there exists a substantial agreement between pap smear cytology and HPE (Table 2).

### Comparison of VIA and HPE findings

Out of 210 cases of cervical lesions, a total of 114 cases were diagnosed as neoplastic cervical lesions on both HPE and VIA examinations, which were considered as TP. On VIA examination, 36 cases were misdiagnosed as neoplastic cervical lesions, which were then correctly diagnosed as non-neoplastic cervical lesions by HPE. Hence these 36 cases were considered as FP. A total of 45 cases were diagnosed as non-neoplastic cervical lesions on both HPE and VIA examinations. These 45 cases were considered as TN. On VIA examination 15 cases were misdiagnosed as non-neoplastic cervical lesions, which were then correctly diagnosed as neoplastic cervical lesions by HPE and were considered as FN (Table 3).

In the present study, diagnostic sensitivity and specificity of VIA examination in detecting various cervical lesions were found to be 88.37% and 55.55%, respectively. PPV was found to be 76% and NPV was found to be 75%. The overall diagnostic accuracy of VIA examination in detecting neoplastic cervical lesions was found to be

75.71%. Cohen’s kappa (κ) value was found to be 0.462, which indicates moderate agreement between VIA and HPE (Table 3).

## DISCUSSION

Women of the menstrual and postmenopausal age group suffer from various cervical problems ranging from mild infections and inflammations to malignant cervical cancer, which require early detection by an effective screening program.<sup>6</sup> Under the “revised 2014 Bethesda system for reporting of cervical cytology,” mild infections and inflammations of cervix are classified as NILM.<sup>5</sup> The previous researchers reported that infection is the most common cause of non-neoplastic cervical lesions categorized under NILM.<sup>6,7</sup> In our study, 41.42% pap smears showed no evidence of cellular atypia, and hence were categorized under NILM. This finding is consistent with the study done by Sachan et al.,<sup>8</sup> and Singh et al.<sup>9</sup>

Nayar and Wilbur<sup>5</sup> reported ASC-US as cervical lesions with mild squamous intra-epithelial abnormality falling short of the definitive criteria for diagnosis of LSIL or HSIL. In our study, we found 11.42% smears showing features of ASC-US. In contrast to our findings, Bamanikar et al.,<sup>7</sup> (2.94%), Lahari and Bharathi<sup>10</sup> (4.4%), and Goel<sup>11</sup> (1.9%) reported

lower prevalence of ASC-US, while Singh et al.,<sup>9</sup> (33.34%) and Flyyih et al.,<sup>12</sup> (26.4%) reported a higher prevalence of ASC-US. Some previous researchers reported ASC-US as the commonest pre-malignant cervical lesion.<sup>7,9,13</sup> In contrast to above studies, we found LSIL to be the most common pre-malignant cervical lesion, which is in agreement with the results of Lahari and Bharathi<sup>10</sup> and Nair et al.<sup>6</sup> These differences may be due to awareness of the study population regarding sexual health and timing of the screening test.

Various researchers had done studies to evaluate the efficiency of pap smear cytology for the diagnosis of non-neoplastic and neoplastic cervical lesions.<sup>14,15</sup> In the current study, sensitivity of the pap smear was found to be 90.16%, which indicates pap smear was correctly able to diagnose 90.16% cases of neoplastic cervical lesions, while the specificity was found to be 85.22%, which indicates its ability to correctly diagnose 85.22% cases of non-neoplastic cervical lesions. Our study also revealed PPV and NPV of the pap smear was 89.43% and 86.2%, respectively (Table 2). These findings are in line with the study done by the previous researchers (Table 4).<sup>4,7,11,16</sup> Our study also showed that pap smear was accurately able to diagnose 88.09% of cervical lesions. Similar to our findings, Niyodusenga et al.,<sup>4</sup> Selvanayaki and Archana<sup>16</sup> and Bamanikar et al.<sup>7</sup> found the diagnostic accuracy as 86.86%, 87%, and 89.5%, respectively, but Goel<sup>11</sup> found a higher

**Table 3: Comparison of VIA findings with histopathological findings in diagnosis of cervical lesions (n=210)**

		Histopathological diagnosis		
		Neoplastic (+)	Non neoplastic (-)	Total
VIA findings	Neoplastic (+)	114 (TP)	36 (FP)	150
	Non neoplastic (-)	15 (FN)	45 (TN)	60
Total		129	81	210
Interpretation of the VIA as a screening test		Sensitivity=TP/(TP+FN) = 88.37% Specificity=TN/(TN+FP) = 55.55% Positive Predictive Value (PPV) =TP/(TP+FP) =76% Negative Predictive Value (NPV) =TN/(TN+FN) =75% Diagnostic Accuracy= (TP+TN)/(TP+FN+FP+TN) = 75.71% Cohen’s Kappa value (κ) = 0.462		

**Table 4: Comparison of diagnostic value of Pap smear as screening test for cervical lesions with previous study**

Diagnostic parameter	Pap screening test results of previous study				
	Niyodusenga et al. <sup>4</sup> (2020)	Selvanayaki and Archana <sup>16</sup> (2020)	Goel <sup>11</sup> (2019)	Bamanikar et al. <sup>7</sup> (2016)	Present study
Sensitivity	80.45%	97.2%	87.5%	89.47%	90.16%
Specificity	91.89%	74%	98.9%	88.7%	85.22%
PPV	88.6%	-	94.9%	82.92%	89.43%
NPV	85.71%	-	97.1%	89%	86.2%
Diagnostic accuracy	86.86%	87%	96.7%	89.5%	88.09%

(96.7%) diagnostic accuracy of pap smear cytology for various cervical lesions (Table 4).

Several studies were also conducted to prove the potential value of VIA for the screening of cervical lesions as the primary approach to detect pre-malignant cervical lesions.<sup>4,17,18</sup> Current study found sensitivity and specificity of VIA for the detection of cervical dysplasia to be 88.37% and 55.55%, respectively, while PPV and NPV were found to be 76% and 75%, respectively. Diagnostic accuracy of VIA was found to be 75.71% (Table 3). These findings are in accordance with the previous similar studies (Table 5).<sup>4,18</sup>

Our study showed substantial degree of agreement between pap smear cytology and histopathology for the diagnosis of various cervical lesions with Cohen's kappa ( $\kappa$ ) value being 0.755 (Table 2), whereas a moderate degree of agreement was found between VIA and histopathology for the diagnosis of various cervical lesions with Cohen's kappa ( $\kappa$ ) value being 0.462 (Table 3). This indicates that pap smear is a better screening tool as compared to VIA for the screening of women with cervical lesions, but VIA is a simpler, faster, and cost-effective tool in comparison to pap smear cytology and histopathology.

Cervical carcinoma has an indolent course of progression from a pre-invasive to an invasive cervical cancer.<sup>12</sup> Our study revealed that diagnostic accuracy of VIA (75.71%) for the diagnosis of cervical lesions was lower in comparison to pap smear cytology (88.09%). The present study also showed that diagnostic accuracy of pap smear cytology for the diagnosis of non-neoplastic cervical lesions was 85.22% (75/88), whereas for pre-invasive cervical lesions it was 89.91% (107/119) (Table 1). This indicates that VIA and pap smear can be used as an effective and non-invasive screening tools for the detection of cervical lesions at an early stage. If detected early, these lesions can be managed accordingly increasing the survival of women and thus, decrease of the burden of cervical cancer.

**Table 5: Comparison of diagnostic values of VIA as screening test for cervical lesions with the previous study**

Diagnostic parameter	VIA screening results of previous study		
	Niyodusenga et al. <sup>4</sup> (2020)	Bhattacharyya et al. <sup>18</sup> (2015)	Present study
Sensitivity	88.5%	89%	88.37%
Specificity	84.68%	87%	55.55%
PPV	81.91%	-	76%
NPV	90.38%	-	75%
Diagnostic accuracy	86.36%	87%	75.71%

Liquid-based cytology (LBC) outperforms the traditional pap smear approach in detecting squamous cell carcinoma recurrence, according to Singh et al.<sup>19</sup> Various researches revealed that switching from conventional pap smear to LBC reduced the percentage of unsatisfactory samples.<sup>19,20</sup> This discrepancy could be due to the fact that with LBC, all drying artifacts such as cytolysis are minimized due to the cells' immersion in the liquid fixative. Conventional pap smears are insufficient due to thick smear, which is not a problem for LBC, because of equal dispersion of cells in the liquid media. The number of cells also increases with LBC since cytobrush sampling is used.

### Limitations of the study

The present study has few important limitations. LBC procedures may reduce the number of unsatisfactory smears even further and also better method of cytological follow up for post-treated patients with cervical cancer.<sup>19</sup> LBC was not done in our study, because they were not cost-effective in our setup. Our study was unable to investigate psycho-social and cultural risk variables in depth. More research into the causes why those who do not participate in screening programs may be beneficial.

## CONCLUSION

We concluded that pap smear and VIA have a high sensitivity with a good diagnostic accuracy for the detection of neoplastic cervical lesions, which indicates that they can be used as an efficient screening tool for the detection of cervical lesions in their earlier stage. Since cervical cancer is one of the major causes of cancer-associated morbidity and mortality in India, early detection of this lesion in its pre-malignant stages can provide a better outcome owing to timely management of patients by clinician's, thereby increasing the quality of life and survival of the patients.

## ACKNOWLEDGMENT

Our authors would like to thank all the patients participating in our study. Furthermore, we would like to thank all laboratory and paramedical staff for their diligent dedication and hard work in a pathology laboratory.

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**Author's Contributions:**

**RJ-** Concept and design of the study, interpreted the results, prepared first draft of manuscript; **JJ-** Interpreted the results, reviewed the literature and manuscript preparation; **AJ-** Study design, statistical analysis and interpretation, and finalization of the draft manuscript.

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**Source of Funding:** None, **Conflicts of Interest:** None.