

## Comparison of Visual Inspection with Acetic Acid (VIA) and Liquid Based Cytology (LBC) with Reflex HPV DNA Test in Cervical Cancer Screening

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### Abstract

A cross-sectional study was conducted to examine the diagnostic outcome of visual inspection with acetic acid (VIA) and liquid based cytology (LBC) and see the association of VIA and LBC with HPV status, between March 2015 and June 2017. A total of 72 women attending the Out-Patient Department (OPD) of Gynaecology (Colposcopy Clinic) of Bangabandhu Sheikh Mujib Medical University (BSMMU) for VIA test participated in the study. All the participants underwent both VIA and LBC procedures first. Then HPV DNA test was done in all the cases by hybrid capture (hc2 High Risk HPA DNA test) from residual LBC samples. Thereafter, biopsies were taken from all the patients for histopathological confirmation. The mean age of the participants was 40.22±12.29 years, (age range 18-72). Cervical lesions of total 36 patients were VIA positive. Among them 2(5.6%) patients were HPV positive and 34(94.4%) were HPV negative. There was no significant association found between VIA positivity and HPV positivity ( $p>0.05$ ). Based on LBC, ASCUS were found in 4 patients, NILM in 52 patients and LSIL in 2 patients; all were HPV negative. ASC-H were found in 4 cases among them 1(25.0%) was HPV positive and 3(75.0%) were HPV negative. Squamous cell carcinoma was found in 1, which was HPV positive. The association between two groups was statistically significant ( $p<0.05$ ). We found the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of (VIA), as compared against histopathology, were 75.0%, 40%, 45.8%, 20% and 88.9% respectively, while for liquid based cytology (LBC) those were found 75.0%, 90.6%, 88.9%, 50.0% and 96.6% respectively. Our data suggest that liquid based cytology (LBC) with reflex HPV testing gives better results than conventional screening through VIA test.

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**Key words:** Cervical cancer, screening, visual inspection using acetic acid (VIA), liquid based cytology (LBC), HPV DNA test.

### Introduction

Cervical cancer is the most common reproductive cancer in women in Bangladesh, and most women come for diagnosis and treatment when it is too late.<sup>1</sup> That means cervical cancer is preventable if detected at an early stage. Interestingly, early detection of cervical cellular changes and cervical intraepithelial neoplasia (CIN) followed by appropriate treatment will reduce the risk of developing cancer.<sup>2,3</sup> In Bangladesh, government strategy to screen for cervical cancer is visual inspection with acetic acid (VIA) based projects, which have been running over a decade.<sup>4</sup> However, depending solely on VIA status may lead to over treatment like more biopsies than necessary and the role of cervical cytology in VIA positive women has not been well documented.<sup>5</sup>

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American Cancer Society (ACS) recommends that women receive one of two options for testing for cervical cancer: PAP smear or Liquid Based Cytology (LBC) and HPV (Human Papilloma Virus) testing.<sup>6,7</sup> Liquid based cytology represents the first major change in preparation method for cervical screening samples for over 50 years. Instead of cells being smeared onto a glass slide, they are washed into a vial of liquid and filtered, and a random sample is presented in a thin layer on a glass slide. These slides can then either be screened by skilled staff or subjected to partially automated imaging. The process is being widely used in the UK, United States, many European countries, and elsewhere.<sup>8</sup> Besides, a high Prevalence of cervical cancer in developing countries is related to many risk factors such as early marriage, early starting of sexual activity, multiparity, low socioeconomic condition and high incidence of sexually transmitted diseases (STD) and Human Papilloma Virus (HPV) infection.<sup>9</sup> Several studies have established HPV infection as a major risk factor for development of Cervical Intraepithelial Neoplasia (CIN) and Invasive Cervical Cancer (ICC).<sup>10,11</sup> Research also showed that over 90% of cervical cancers worldwide contained HPV DNA. Epidemiological and molecular biological studies have shown that persistent infection with high-risk HPV is necessary in the pathogenesis of cervical cancer.<sup>10</sup>

To our knowledge, no study has examined or compared the diagnostic outcome of liquid based cytology (LBC) and conventional visual inspection with acetic acid (VIA) and test the association of LBC or VIA with HPV status in Bangladesh to date. The present study aims at preparing cervical cytology smears using liquid based cytology (LBC) and to compare the results with that of conventional VIA test and see the association of LBC and VIA with HPV status.

## Methods

This cross-sectional study was conducted between March 2015 and June 2017. The study population included women attending the Out-Patient Department (OPD) of Gynaecology (Colposcopy Clinic) of Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital for VIA test.

Exclusion criteria included who do not give consent, pregnant women, patients with massive vaginal bleeding and patients of treated cervical carcinoma. Finally, a total 72 women were included in this study. The results of VIA method and Liquid-based cytology method were observed by two independent observers in Department of Pathology of the same institution.

At first, a Cusco's bivalve speculum was introduced under good lighting to visualize the cervix. Then 5% acetic acid solution was applied to cervix using a cotton swab and after 1 min visual inspection of cervix was done for the development of any acetowhite area near squamocolumnar junction or close to the external os or presence of aceto-white growth. The VIA results were interpreted as positive or negative by one of the investigators. Thereafter, endocervical broom brush (supplied by Becton Dickinson) was used to obtain sample. Brush was rotated against the ectocervix for a 3600 rotation to include the transformation zone. Material on the brush was smeared onto a glass slide for conventional smear preparation and fixed in alcohol. The brush along with remaining material was detached and rinsed into a bottle containing liquid fixative containing 24% ethanol. Liquid based cytology was done by Beckton Dickinson Sure-path technique. Here, the cells were prepared at a monolayer by separating on concentration gradient with the help of sure path preparation made which produces a 13 mm<sup>2</sup> area of representative sample on the slide. It was then manually

stained by stains supplied by Beckton Dickinson. Then HPV DNA test was done in all the cases by hybrid capture (hc2 High Risk HPA DNA test) from residual LBC samples. Thereafter, biopsies were taken from all the patients for histopathological confirmation.

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Chi-Square test was used to analyze the categorical variables, shown with cross tabulation. Sensitivity, specificity, positive predictive value and negative predictive value for liquid-based cytology and conventional Pap smears were calculated. P value of <0.05 was taken as significant. The study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

## Results

In the present study, the mean age of the patients was 40.22±12.29 years, with a range between 18 and 72 (Table-I). Cervical lesions of total 36 patients were VIA positive. Among them 2(5.6%) patients were HPV positive and 34(94.4%) were HPV negative. There was no significant association found between VIA positivity and HPV positivity ( $p>0.05$ ). This result suggests a poor sensitivity of VIA test (Table-II). Based on LBC, in the study respondents, ASCUS were found in 4 patients; all were HPV negative. NILM were found in 52 patients; all were HPV negative. LSIL were found in two cases and both were HPV negative. ASC-H were found in 4 cases among them 1(25.0%) was HPV positive and 3(75.0%) were HPV negative.

Squamous cell carcinoma was found in one and it was HPV positive case. The association between two groups was statistically significant ( $p<0.05$ ) (Table-III). We found the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of (VIA), as compared against histopathology, were 75.0%, 40%, 45.8%, 20% and 88.9% respectively. In contrast, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of liquid based cytology (LBC) were found 75.0%, 90.6%, 88.9%, 50.0% and 96.6% respectively (Table-IV).

**Table-I: Distribution of the study respondents by age (N=72)**

| Age (in year) | Frequency    | Percentage (%) |
|---------------|--------------|----------------|
| ≤30           | 18           | 25.0           |
| 31-40         | 26           | 36.1           |
| 41-50         | 16           | 22.2           |
| 51-60         | 6            | 8.3            |
| >60           | 6            | 8.3            |
| Mean ±SD      | 40.22 ±12.29 |                |
| Range         | 18-72        |                |

**Table-II: Association between VIA test of cervical lesion with HPV DNA status (N=72)**

| VIA             | HPV            |      |                 |       |
|-----------------|----------------|------|-----------------|-------|
|                 | Positive (n=2) |      | Negative (n=61) |       |
|                 | n              | %    | n               | %     |
| Positive (n=45) | 2              | 4.44 | 43              | 95.56 |
| Negative(n=27)  | 0              | 0.0  | 27              | 100.0 |

At 5% level of significance against df = 1, P value was >0.05; P value reached from McNemar Test.

**Table-III: Association between LBC of cervical smear with HPV DNA status**

| Liquid based cytology | HPV      |       |           |       | P value           |
|-----------------------|----------|-------|-----------|-------|-------------------|
|                       | Positive |       | Negative  |       |                   |
|                       | N        | %     | N         | %     |                   |
| NILM (n=52)           | 0        | 0.0   | 52        | 100.0 | 0.02 <sup>S</sup> |
| ASCUS (n=4)           | 0        | 0.0   | 4         | 100.0 |                   |
| LSIL (n=2)            | 0        | 0.0   | 2         | 100.0 |                   |
| ASC-H (n=4)           | 1        | 25.0  | 3         | 75.0  |                   |
| SQ.C.C (n=1)          | 1        | 100.0 | 0         | 0.0   |                   |
| <b>Total</b>          | <b>2</b> |       | <b>61</b> |       |                   |

S=significant; P value reached from Fisher exact test; NILM- Negative for Intraepithelial lesion or malignancy; ASCUS- Atypical Squamous Cells of Undetermined Significance; LSIL- Low grade Squamous Intraepithelial Lesions; ASC-H-Atypical Squamous Cells-cannot exclude HSIL; SQ.C.C-Squamous cell carcinoma.

**Table-IV: Sensitivity, specificity, accuracy, positive and negative predictive values of VIA test and LBC, as compared with histopathology (N=72).**

| Validity test             | VIA Test | LBC Test |
|---------------------------|----------|----------|
| Sensitivity               | 75.0     | 75.0     |
| Specificity               | 40.0     | 90.6     |
| Accuracy                  | 45.8     | 88.9     |
| Positive predictive value | 20.0     | 50.0     |
| Negative predictive value | 88.9     | 96.7     |

## Discussion

In this present study, the majority 26(36.1%) of the patients were in 31-40 years age group. The mean age was 40.22±12.29 years with range from 18 to 72 years. Rahman *et al.* had 68 patients aged between 25 and 70 years (mean: 41.4±10.6 years).<sup>12</sup> Karimi-Zarchi *et al.* showed that the mean age was 42±9.94 years, and ranged between 21 to 70 years of age, having 150 patients in total.<sup>13</sup> Moy *et al.* showed the enrolled women had a mean age of 39±5.6 years.<sup>14</sup> Those findings were almost similar to our study.

Our study showed more than half (54.2%) patients were found VIA positive and 33(45.8%) were VIA negative. Nessa *et al.* showed that out of 104098 women screened, 5013 (4.8%) were positive on VIA, while Nuranna *et al.* found that out of 25,406 women, there were 1,192(4.7%) of VIA positive cases.<sup>15,16</sup> The high geographic variability in the prevalence of cervical inflammation and the inability to identify a responsible infectious agent could be expected to contribute to poor reproducibility of VIA performance across broad populations.<sup>17</sup>

The present study showed among LBC of the study population it was observed that majority (83.3%) patients were found NILM, five (6.9%) ASC-H, four (5.6%) ASCUS, two (2.8%) LSIL, one (1.4%) Squamous cell carcinoma. Arunratsamee and Siwadune showed that out of 250 women 93(37%) were NILM, 20(8%) were ASCUS, 7(2.8%) were ASC-H, 49(19.6%) LSIL and 11(4.4%) cases of squamous cell carcinoma.

They included previously diagnosed cases of abnormal Pap smear purposefully since shows higher ranges of positive findings compared to our study.<sup>18</sup> Karimi-Zarchi *et al.* showed out of 150 patients, nine (7%) patients in LBC, while incidence of cervicitis were found in six patients (4%) in LBC.<sup>13</sup>

Our LBC demonstrated high rates of cervical squamous intra-epithelial lesions than that of VIA. Similar results were found by Kirschner *et al.*, Nandini *et al.* and Tesfaw *et al.*<sup>19-21</sup>

We found the sensitivity and specificity of (VIA), as 75.0% and 40%, while the same for the liquid based cytology (LBC) were 75.0% and 90.6% respectively. Surprisingly, Nurunnabi & Sultana reported the sensitivity and specificity of VIA as 94.4% and 97.87% respectively, which is of higher value than that of ours.<sup>22</sup> Prevalence of positive VIA test depends on the characteristic of the population studied e.g., asymptomatic women or symptomatic; co-incidental pathology of cervical dysplastic lesions and cervicitis or inflammation; cervical anatomy or area of transformation zone which is affected by age or menopausal status; or parity.<sup>23</sup> Overall, our data suggest that LBC with reflex HPV DNA test is a better screening procedure than that of VIA with reflexive HPV DNA test LBC alone, which is also supported by Mandelblatt *et al.*<sup>24</sup> However, in Bangladesh, having a low resource setting, we need to consider the cost effectiveness of LBC with reflex HPV screening, specially in the absence of the facility for reflex HPV DNA testing in majority of centres. This study provides an important insight on current patterns VIA screening as well as the superiority of the LBC with reflex HPV screening in women for cervical cancer screening.

## Conclusion

Our data suggest that liquid based cytology (LBC) with reflex HPV testing gives a better result than conventional screening through visual inspection using acetic acid (VIA) test. However, we recommend further research in large scales in multicentre, with larger population from both urban and rural areas as well as advanced diagnostic facilities.

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