TAJ December 2009; Volume 22 Number 2

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Role of VIA and PAP Smear in the Diagnosis of Cervical Precancers : A Study of 115 Cases

R A Jahan[1], F Rahman[2], S M Badruddoza[3], T A Nasir[4], A N N U Ahmed[5], M Kamal[6]

Abstract

Carcinoma of cervix is the most frequent neoplasm of female genital tract. In Bangladesh cervical cancer is the commonest malignancy of women. This high incidence of cervical cancer is attributed to the lack of screening program, particularly in the women of low socio-economic status. Invasive cervical cancer is preceded by a long premalignant phase known as cervical intraepithelial neoplasia (CIN). The goal of cervical cancer screening is the detection and treatment of precancer before cancer develops. The aim of this study was to evaluate the test parameters using visual inspection with acetic acid (VIA) and cervical cytology in screening and early diagnosis of the precancerous lesions of cervix. This study deals with 115 cases from Gynecology Outpatient Department of Rajshahi Medical College, Rajshahi from July 2006 to June 2008. After vaginal examination Pap smear was collected, followed by VIA and punch biopsy of cervix. All the data were evaluated by standard statistical methods. The sensitivity of VIA was 94.11% while Pap smear was 64.7%. The specificity of Pap smear was 93.94% while VIA was 57.57%. These two tests may be considered as a suitable early detection technique in the developing countries where other test like HPV-DNA detection is a costly one.

TAJ 2009; 22(2): 183-189

Introduction

Cervical cancer is often the most common cancer in women and may constitute up to 25% of all female cancers in developing countries[1]. It affects nearly half a million women each year worldwide, claiming death toll of a quarter of a million lives i.e., 50% mortality rate[2]. In the United States, an estimated 9710 new cases of invasive cervical cancer are diagnosed annually and there are 3700 deaths from the disease; this represents 1.3% of cancer deaths of women[3].

Bangladesh and India have an annual incidence of cervical cancer 11956 and 125952 respectively[4]. It constitutes about 24.6% among total female cancer in Bangladesh[5].

The first screening method for cervical cancer i.e., Pap smear technique relies on a microscopic examination of cervical cells collected during the cervical smear procedure. This method discovered by George N. Papanicolaou in 1928 allows for detection of cellular changes indicating the possible genesis of cervical cancer.

Visual inspection of cervix with acetic acid (VIA) is another screening test. Various studies show that VIA is simple, accurate, cost effective and acceptable screening method to most women[6].

[1] Lecturer, Department of Pathology, Rajshahi Medical College, Rajshahi.
[2] Professor & Principal, Department of Pathology, Anwar Khan Modern Medical College, Dhaka.
[3] Professor, Department of Pathology, Rajshahi Medical College, Rajshahi.
[4] Professor, Department of Pathology, Pathology and Lab Coordinator, Apollo Hospital, Dhaka.
[5] Professor, Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka.
[6] Professor, Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka.

Material and Methods

This cross sectional study was carried out in the Department of Pathology, Rajshahi Medical College, Rajshahi, during the period from July 2006 to June 2008. A total of 115 patients of 21 to 60 years age group were selected from patients attending the Gynecology and Obstetrics Out Patient Department of Rajshahi Medical College Hospital, Rajshahi.

Patients were enrolled on the basis of history, physical examination, inclusion and exclusion criteria. The inclusion criteria include women aged 21 - 60 years, having the history of pervaginal discharge, irregular pervaginal bleeding, postcoital bleeding, lower abdominal pain, back pain and clinically unhealthy looking cervix.

The exclusion criteria include histopathologically proven cases of cervical lesions or with history of hysterectomy, age more than 60 years, age less than 21 years, pregnant, menstruating women and unmarried women.

Method of data collection

Data were collected from the enrolled patients by using a questionnaire. After recording clinical history, cervix was examined on naked eye by cuscos speculum. Pap smears and VIA tests were done concurrently. Subjects with positive VIA or positive Pap smear and subjects with clinical suspicion even with negative screening tests underwent colposcopy directed biopsy. A total of 50 patients were selected for biopsy. Clinical history, physical findings, Pap smear findings, VIA findings, and histological findings were recorded in the pre-designed patient's profile made for the study.

Technique of Pap smear preparation

The cervical scrape smear was the specimen used for screening precancerous lesions. Before collection a clean dry glass slide was numbered. The small end of the wooden Ayre's spatula was placed through the external os high into the canal. The spatula was then rotated clockwise at 360 degree angle thoroughly for scraping the entire cervical os. The collected samples were spread on 2/3rds of clean glass slides, which were immediately dipped into Coplin jar containing fixative (95% ethyl alcohol) for at least 30 minutes. Then the smears were stained by modified Papanicolaou staining method for cytological diagnosis. Pap smears were evaluated and diagnosed using the Bethesda (2001) system.

Technique of Visual inspection of cervix with acetic acid (VIA)

After obtaining the specimen for cytology, a solution of 5% acetic acid was applied to the cervix with cotton tipped applicator for one minute. After which the cervix was examined in good light. Abnormal cells temporarily turn white and reveal aceto-white epithelium on the cervix. Acetowhitening of abnormal cervical epithelium has been suggested as an indicator of increased cervical cancer risk[7]. Detection of well defined, opaque aceto-white lesions close to the squamocolumnar junction, well defined circumferential aceto-white lesions; or dense aceto-whitening of ulceroproliferative growth on the cervix constituted positive VIA. In absence of these changes the test was interpreted as negative.

Technique of histopathological examination

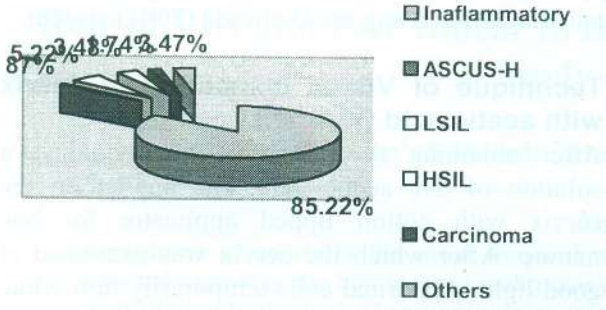
Biopsies were done in 50 patients. Gross examination of the specimens were done and embedded as such. Routine tissue processing with paraffin impregnation was done. For microscopic examination routine paraffin sections were stained with haematoxylin and eosin.

Results and observations

Pap smear cytopathological diagnosis

Of the 115 cases satisfactory smears were obtained in 113 (98.26 %) cases and 2 (1.74 %) smears were unsatisfactory which were included in other diagnostic criteria. On cytological examination 98 (85.22%) were diagnosed as "Inflammatory/ Negative for intraepithelial lesions or malignancy". One (0.87%) was diagnosed as ASCUS-H, 6 (5.22%) were as Low-grade squamous intraepithelial lesion (LSIL), 4 (3.48%) were High-grade squamous intraepithelial lesion (HSIL, Figure-1). 2 (1.74%) were found to be of squamous cell carcinoma. Remaining 4 (3.47%), 2 (1.74%) were unsatisfactory and 2 (1.74%) were

inflammatory which were included in other diagnostic finding. These were shown in the following Pie diagram:



Pie diagram: Showing Pap smear cytological diagnosis of 115 cases

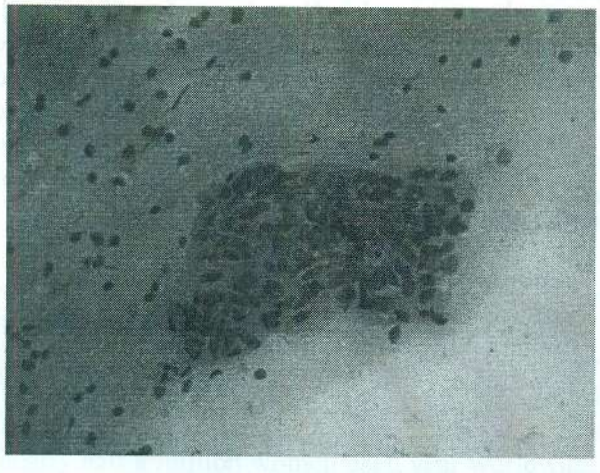


Figure-1: Cervical smear showing HSIL, (Papanicolaou stain x 400), case no 17.

VIA findings

Among 115 patients included in this study subsequent VIA examination showed 30 positive cases (Figure-2) and 85 negative cases (Table - 1).

Table-1: VIA test findings of 115 cases

VIA findings	Frequency	Percent
Positive	30	26.09
Negative	85	73.91
Total	115	100.00

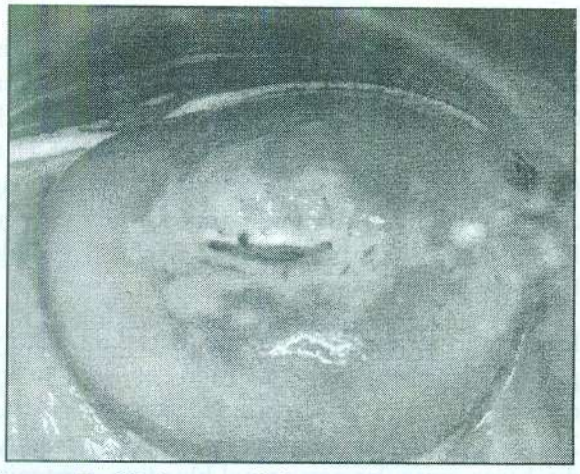


Figure-2: VIA test of the cervix showing dense acetowhite area (Colposcopy x 7.5), case no 17.

Histopathological findings

Out of 115 cases in the study, biopsy specimens were taken from 50 patients. In 65 (54.8%) cases biopsies were not done, of which 63 were normal on screening tests and 2 refused biopsy. On histopathological examination, 31 (62%) were diagnosed as chronic cervicitis, 9 (18%) as CIN-I (Figure -3), 1 (2%) as CIN-II, 4 (8%) were CIN-III (Figure-4) and invasive squamous cell carcinoma was diagnosed in 3 (6%) patients. Two (4%) cases were diagnosed as endocervical polyp and chronic cervicitis with squamous metaplasia, which were included in other diagnostic finding. Table-2 showed the distribution of histopathological diagnosis of 50 cases.

Figure-3: Histological section of cervix showing CIN-I / LSIL, (H & E stain x 400), case no 31.

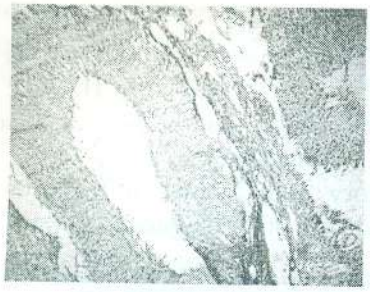


Figure-4: Histological section of cervix showing CIN-III / HSIL, (H & E stain x 400), case no 17.

Table-2. Histopathological findings of 50 cases

Histopathological findings	Frequency	Percent
Inflammatory	31	62
CIN-I	09	18
CIN-II	01	2
CIN-III	04	8
Carcinoma	03	6
Others	2	4
Total	50	100.0

Pap smear cytology and its relation with histopathology

Among the 31 cases of chronic cervicitis, 29 (93.6%) cases were diagnosed as inflammatory cytology, 1(3.2%) was diagnosed as ASCUS-H and 1 as LSIL. Out of 9 cases of CIN-1 lesion, 5 (55.6%) were diagnosed as LSIL and 4 (44.4%) were diagnosed as inflammatory cytology. Out of 5 CIN-II/III cases, 4 (80%) were diagnosed as HSIL (Figure-4), 1 (20%) as inflammatory cytology. Among the 3 squamous cell carcinoma, 2 (66.7%) were diagnosed as carcinoma and 1(33.3%) was inflammatory. Remaining 2 (100%) were evaluated as inflammatory cytology. Table-3 showed the relation of Pap smear cytology with biopsy.

Table-3: Findings of Pap smear in relation to histopathology

Histopathological diagnosis	Number of patients	Pap smear cytological diagnosis				
		Inflammatory cytology	ASCUS	LSIL/CIN-I	HSIL/CIN-II-III	Squamous cell carcinoma
Chronic cervicitis	31	29 (93.6%)	1(3.2%)	1 (3.2%)	-	-
CIN-I	9	4 (44.4%)	-	5 (55.6%)	-	-
CIN-II/III	5	1 (20%)	-	-	4 (80%)	-
Invasive squamous cell carcinoma	3	1 (33.3%)	-	-	-	2 (66.7%)
Others	2	2 (100%)	-	-	-	-
Total	50	41	1	2	4	2

False positive = 2 (Discase negative but test positive)
False negative = 6 (Disease positive but test negative)
True positive = 11 (Those who are both test positive and disease positive)
True negative = 31 (Those who are both test negative and disease negative)

VIA tests and its relation with histopathology

Of the total 50 cases in which biopsy were done, VIA tests were positive in 30 cases and negative in 20 cases. Out of 31 histopathologically diagnosed cases of chronic cervicitis, 13 (41.94%) were VIA positive and 18 (58.06%) were VIA negative. Out of 9 CIN-I cases, 8 (88.9%) cases were VIA positive and 1 (11.11%) was VIA negative. All 5 (100%) CIN-II/III cases and all 3 (100%) malignant cases were VIA positive. Of the two others diagnostic findings, one was VIA positive and one was VIA negative, these were shown in the Table- 4.

Table-4: Findings of VIA tests in relation to histopathology

Histopathological diagnosis	No. of patients	VIA test results Positive (%)	Negative (%)
Chronic cervicitis	31	13 (41.94)	18 (58.06)
CIN I	9	8 (88.89)	1 (11.11)
CIN II/III	5	5 (100)	-
Squamous cell carcinoma	3	3 (100)	-
Others	2	1 (50)	1 (50)
Total	50	30	20

False positive = 14 (Disease negative but test positive)
False negative = 1 (Disease positive but test negative)
True positive = 16 (Those who are both test positive and disease positive)
True negative = 19 (Those who are both test negative and disease negative)

Diagnostic methods	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Pap	11	31	2	6	64.71	93.94	84.62	83.78	84.00
VIA	16	19	14	1	94.11	57.57	53.33	95.00	70.00

On histopathological examination of 50 biopsy specimens, 31 (62%) cases was diagnosed as inflammatory or chronic cervicitis, 9 (18%) cases as CIN-I, 1 (2%) as CIN-II, 4 (8%) as CIN-III, 3 (6%) as invasive squamous cell carcinoma and 2 (4%) as chronic cervicitis with squamous metaplasia and endocervical polyp. A study carried out by Hussain[8], showed the incidence of inflammation 82.5%, koilocytotic atypia 3%, CIN-I 9.5%, CIN-II 1%, CIN-III 0% and invasive carcinoma 4%, which were comparable to the present study.

On cytological examination 98 (85.22%) were diagnosed as "Inflammatory /Negative for intraepithelial lesions or malignancy". One (0.87%) was diagnosed as ASCUS-H, 6 (5.22%) were as LSIL, 4 (3.48%) were HSIL, 2 (1.74%) cases were found to be of squamous cell carcinoma. Remaining 4 (3.47%), 2 were unsatisfactory and 2 were inflammatory which were included in other diagnostic finding.

A study on Pap smear carried out by Mimi[9], showed inflammatory lesion 82%, inflammation with metaplasia 9%, low-grade lesion 4.4%, high-grade lesion 1.6%, squamous cell carcinoma 2.4%. Here percentage of inflammatory lesion, LSIL and squamous cell carcinoma were almost similar to this study. It differed with this study in case of HSIL.

VIA examination showed 30 (26%) cases positive and 85 (74 %) cases negative. VIA was positive in 41.94% chronic cervicitis, 88.9% CIN-I, all cases of CIN-II/III lesions and squamous cell carcinoma. Begum[10] showed VIA positive in 41% chronic cervicitis, 91% CIN-I and all cases of CIN-II/III lesions and squamous cell carcinoma. This finding showed similarity with the present study. Acetic acid visualization of the cervix can detect dysplasia otherwise missed by Papanicolaou test screening[11].

In this study both Pap smear and VIA were taken as a screening test for cervical lesions. To the outcomes of the present study, the findings were compared with observations of others. Comparing VIA with cytology Gaffikin[12] noted that the overall usefulness of VIA compares favorably with that of the Pap test. Doh[13] reported sensitivity of VIA was 70.4% versus 47.7% for Pap smear and specificity of VIA was 77.6% versus 94.2% for Pap smear. Hussain et al.[8] reported sensitivity, specificity, PPV, NPV for Pap smear cytology were 54.3%, 94.5%, 67.9%, 90.7% and for VIA were 80%, 88.5%, 59.6%, 95.4% respectively.

In this type of study validity refers to its ability to diagnose cases of neoplastic disorders and distinguish them from non-neoplastic conditions. Sensitivity measures how well a test identifies truly ill people and specificity measures how well a test identifies truly well people. Sensitivity will be high if false negative report is low. Specificity

will be high when false positive report is low. In reviewing the observations by different authors it had been seen that sensitivity of Pap smear ranged from 29.6%[14] to 88.6%[15] and specificity of Pap smear ranged from 69.6%[16] to 97.9%[17]. Regarding VIA test sensitivity ranged from 60.5%[18] to 94.12 %[10] and specificity ranged from 30.4%[16] to 88.5%[8].

The study of Pap smear showed that sensitivity and specificity were more or less similar to other observations. This study also showed that VIA sensitivity was in the upper limit of range observed by others and specificity was between the ranges to other observations. The result of comparative study between Pap smear and histopathology was significant (P<0.10), between VIA and histopathology was highly significant (P<0.001).

In explaining the present study it had been shown that after getting a negative Pap smear result, the probability of not having CIN/cancer was 83.78% and the chance of missing CIN/cancer was 16.22%. The PPV and NPV for VIA were 53.53% and 95% in this study. The NPV for VIA in this study also reflects a chance of missing CIN/cancer was 5%. In this study the sensitivity of VIA was 94.11% but specificity was 57.57%. The PPV was only 53.33%, which indicates the high degree of over diagnosis. One of the contributing factors for this high number of false positive may be the low threshold for VIA positive.

Considering the socioeconomic condition and infrastructure of health care system of our country, a two step-screening program for cervical cancer seems to be suitable. VIA has the advantage of being inexpensive and simple to perform, provides an immediate result with a high sensitivity. VIA test of all women after 20 years at every 2-3 years interval, which can be done at the family planning clinic or rural or urban health clinic, by the trained paramedics and nurses. Cytology on the other hand has good specificity and accuracy but has high false negative rate. Combined use of VIA and Pap smear minimize their disadvantages.

In two step screening system the VIA positive patients are to be subjected to a second screening test by Pap smear at a center where cytopathologist is available. So the burden on cytopathology laboratory and on the personnel will be lessened. Once a cytological abnormality is detected, management is predicted on whether the abnormality is CIN or uncertain significance (atypical cells). HPV-DNA testing results can be used as a tool to better determines the need for referrals for colposcopic biopsy, especially for patients with an ASCUS diagnosis[19].

Conclusion

It was observed that the routine Pap smear test was better than that of VIA test as a single screening test. As Pap smear cytology is not so much established cervical screening method in our country due to lack of necessary resources and HPV-DNA test is a costly one. So VIA can be used as an effective cervical screening test. Where facilities are available, the two tests can be used as screening method.

References

1. Burd, E.M. 2003. Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews*. 16: 1 – 17.
2. Parkin, D.M., Pisani, P. and Ferlay, J. 1999. Epidemoilogy, clinical features, and diagnosis of invasive cervical cancer. *CA Cancer J Clin*. 49(1): 33-64.
3. Jemal, A., Siegel, R., Ward, E. *et al.* 2006. Epidemiology, clinical features, and diagnosis of invasive cervical cancer. *CA Cancer J Clin*. 56(2):106-130.
4. Jahan, S, (ed). *et al.* 2007. *Cervical and breast cancer screening programme trainer's handbook*. Department of Gynaecology and Obstetrics, Bangabandhu Sheikh Mujib Medical University, Dhaka.
5. Talukder, H. *et al.* 2005. *Annual Report 2005*. Bangladesh National Institute of Cancer Research and Hospital, Directorate General of Health Services, Ministry of Health and Family Welfare.
6. Carr, K.C., Sellors, J.W. 2004. Cervical cancer screening in low resource setting using visual inspection with acetic acid. *J Midwifery Womens Health*. 49(4): 329 – 337.
7. Ficsor, G., Fuller, S.K., Jeromin, J.L., Beyer, R.D. and Janca, F.C. 1990. Enhancing cervical cancer detecting using nucleic acid hybridization and acetic acid tests. *Nurse Pract*. 15: 326 – 330.

8. Hussain, M., Nasir, T.A. *et al.* 2007. Can VIA replace Pap smear as screening tool for cervical neoplasia: evaluation in 200 cases. *Bang J Pathol.* 22(2), pp. 2 - 9.

9. Mimi, S.A., Hena, B.S.N., Khan, M.A.H. and Bhuiyan, M.S.H. 2002. Pap's smear: A study on Bangladeshi women in Sylhet. *OMTAJ.* 1(1): 4 – 6.

10. Begum, T. 2006. *Correlation between Pap smear cytology and visual inspection with acetic acid in the diagnosis of cervical lesions.* M Phil Thesis, Sylhet MAG Osmani Medical College, Sylhet.

11. Van, L.L.E., Frederik, F., Broekhuizen Jenzer, R., Steele, R.H. et al. 1993. Acetic acid visualization of the cervix to detect cervical dysplasia. *Obstet Gynecol.* 81(2): 293 – 295.

12. Gaffikin, L., Lauterbach, M. and Blumenthal, P.D. 2003. Performance of visual inspection of acetic acid for cervical screening: a qualitative summary of evidence to date. *Obstet Gynecol Surv.* 58(8): 543 – 550.

13. Doh, A.S., Nkele, N.N., Achu, P., Nijru D.S., Essimbi, O., and Nkegoum, B. 2005. Visual inspection with acetic acid and cytology as screening methods for cervical lesion in Cameroon. *Int J Gynaecol Obstet*; 89(2): 167 – 173.

14. University of Zimbabwe and JHPIEGO Cervical Cancer Project. 2003. Visual Inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. *The Lancet.* 353: 869 - 873.

15. Elit, L., Baigal G., Tan, J. and Munkhtaivan, A. 2006. Assessment of two cervical screening methods in Mongolia: cervical cytology and visual inspection with acetic acid. *J Low Genit Tract Dis.* 10(2): 83 – 88.

16. Tayyeb, R., Khawaja, N.P., and Malik, N. 2003. Comparison of visual inspection of cervix and Pap smear for cervical cancer screening. *J Coll Physicians Surg Pak.* 13(4): 201 - 203.

17. Sankaranarayanan, R., Budka, M., and Rajkumar. R. 2001. Effective screening programmes for cervical cancer in low and middle-income developing countries. *Bulletin of the World Health Organization.* 79: 1-17

18. De Vuyst, H., Claeys, P., Njiru, S., Muchiri, L., Steyaert, S. *et al.* 2005. Comparison of Pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography. *Int J Gynaecol Obstet.* 89(2): 120 - 126.

19. Hong, I.S., Marshalleck, J. et al. 2002. Comparative analysis of a liquid-based Pap test and concurrent HPV-DNA assay of residual samples: A study of 608 cases. *Acta Cytologica.* 46: 828 – 834.

All correspondence to:
R A Jahan
Lecturer
Department of Pathology
Rajshahi Medical College, Rajshahi.