

PAP Smears and Acetic Acid Staining and Their Correlation with Histopathology – Original Research Article

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Abstract

The cervix is the most common site for genital tract infections and a target for viral, chemical carcinogens, which leads to invasive carcinoma. Infections are the most common complaints in gynecological practice. With the advent of new drugs for infectious diseases, the emphasis has moved to chronic diseases such as malignant diseases which are becoming important cause of death. The objective of this study was to compare the visual inspection of acetic acid test qualities with Pap smear and biopsy. Out of 500 cases, majority of the cases (232) were Negative for Intra-epithelial lesion or malignancy (NILM), 126 cases were normal smears, 16 cases were Atypical Squamous Cells of Undetermined Significance (ASCUS), 75 cases were Low-grade Squamous Intra-epithelial Lesion (LSIL), 37 cases were High-grade Squamous Intra-epithelial Lesion (HSIL), 14 were carcinoma of the cervix. Pap smears are required not only for the diagnosis and management of the malignant lesions but it is also helpful in identifying the infectious etiologies and treatment in developing countries. They need to be correlated with histopathology for further management. In developing countries, where it is not feasible to introduce pap smear screening of acceptable quality, VIA is alternative low cost method for cervical screening.

Keywords: Acetic Acid, Carcinoma, Cervix, Malignancy, Infections, Histopathology.

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INTRODUCTION

Cancer cervix is a preventable disease and continues to be a cause of great concern to women's health. Universally, cervical carcinoma is the second most common cancer in women after breast cancer, 80 % of cancer cervix seen in developing countries, where it is the commonest cancer in woman. In India it accounts for 25 to 50 % of all malignancies and every year 1, 00,000 new cases of cervical cancers are registered, mostly in late stages [1]. Incidence is very high in rural areas where cancer cervix accounts for more than half of cancers among women and the number of cases grows due to high population rate. The key to reducing cervical cancer morbidity and mortality is early detection, coupled with timely treatment of cervical precancerous lesions. Cervical cytology often referred to as Pap smear is perhaps the most well known of available screening methods. Although, performing a pap test may seem relatively simple, a large number of steps are required to take an adequate smear, process and analyze the specimen and inform patients of the results. If any of these steps are unreliable or logistically burdensome, the entire screening program could breakdown and with it the

potential for any public health benefit. In poorly resourced locations, visual inspection with acetic acid (VIA) has potential advantages over traditional screening techniques. It involves washing of cervix with acetic acid and the inspected by eye for evidence of disease. The main advantages are- a second person is not required for interpretation of results and the patient need not come again to collect the report as there is immediate feedback of test results to the patient and treatment can be provide immediately after the test.

AIM OF THE STUDY

The objectives of this study are 1) to know the sensitivity and specificity of visual inspection with acetic acid (VIA) in picking up pre-invasive or invasive cancer in an abnormal cervix, 2) to compare the visual inspection of acetic acid test qualities with Pap smear and biopsy, 3) to assess the combined accuracy when using VIA as an adjunct to pap smear.

MATERIALS AND METHODS

This study was done between 2005 and 2007 with total 500 cases. Women between age 20 and 70 years who attended gynecology outpatient department

at KGH, Visakhapatnam with various complaints like leucorrhoea, bleeding per vaginum and other complaints pointing towards cervical diseases. After taking a detailed history and consent for the screening, the data was collected and patients were examined. First an unaided visual inspection of the cervix is performed under good illumination and normal or abnormal features noted. The cervix was visualized with a speculum, Ayre's spatula was inserted in a way that long end goes into cervical canal while smaller end of spatula rests on the ectocervix. Spatula is then rotated through 360 degrees maintaining contact with ectocervix. Care was taken to avoid hemorrhagic artefacts on smeared slides [2-4]. The specimen is immediately spread evenly onto a previously marked glass slide which is immediately fixed in a coplin jar filled with 95% isopropyl alcohol. In some cases aerosol fixative spray was used. After obtaining the pap smear the cervix is painted with 3 to 5% freshly prepared acetic acid solution using sterile cotton swabs. The cervix is inspected after 1 minute and the results were noted as either positive, if there were distinct aceto-white areas or pale white areas, negative if no aceto-white areas were seen. A biopsy was obtained in cases which showed a positive VIA. This is used as the reference standard for the comparison of the two

screening procedures in the study. Slides that have been fixed and stained were screened in a proper manner. The primary screening took place with an objective lens 10x. The screening was systematic and each field of the smear covered in an overlapping manner. The screening was either vertical or horizontal. Vertical screening was superior because it provided better control of overlapping fields. The biopsy specimens were fixed in 10 % neutral buffered formalin. Sections were processed and stained by routine Hematoxylin and eosin stains.

Sampling was not done during menses, and in patients with vaginal contraceptives, vaginal medications for at least 48 hours prior to taking the smears. Sexual abstinence considered was about 24 hours. Post-partum smears were taken only after 6-8 weeks of delivery.

RESULTS

This study was done between 2005 and 2007. A total number of 500 cases were studied. The cases were first classified depending on the age group (Table-1). Acetic acid test was done for all the 500 cases, 358 cases (71.60 %) were tested positive (Table-2).

Table-1: Cases Distribution According To Age

AGE	NUMBER OF CASES	PERCENTAGE
20-30 years	210	42.00 %
30-40 years	160	32.00%
40-50 years	90	18.00%
50-60 years	28	5.60 %
60-70 years	12	2.40 %
TOTAL	500	100 %

Table-2: Acetic Acid Test Results

NUMBER OF CASES	RESULTS	PERCENTAGE
142	NEGATIVE	28.40 %
358	POSITIVE	71.60 %
TOTAL	500	100 %

In 500 cases, 232 cases were observed as inflammatory smears. 14 (2.8%) cases were malignancies (Table-3).

Table-3: Pap Smear Results

NUMBER OF CASES	RESULTS	PERCENTAGE
126	NORMAL SMEAR	25.2 %
232	INFLAMMATORY SMEAR	46.4 %
16	ASCUS	3.2 %
75	LSIL	15 %
37	HSIL	7.4 %
14	CARCINOMA	2.8 %
TOTAL =500		100 %

*ASCUS =Atypical Squamous Cells of Undetermined Significance

*LSIL=Low grade Squamous Intraepithelial Lesion

*HSIL=High grade Squamous Intraepithelial Lesion

Biopsy was done for 358 cases, 14 cases were malignancies (Table-4). Out of 14 malignancies, 4 cases

were large cell keratinizing squamous cell carcinoma and one case was adenocarcinoma.

Table-4: Biopsy Diagnosis Results

NUMBER OF CASES	RESULTS	PERCENTAGE
230	CHRONIC CERVICITIS	64.25 %
68	LSIL	18.99 %
44	HSIL	12.29 %
02	CARCINOMA IN-SITU	00.56 %
14	CARCINOMA	03.91 %
TOTAL = 358		

Table-5: Histological Type of Carcinoma

HISTOLOGICAL TYPE	NUMBER OF CASES	PERCENTAGE
Large Cell Non-Keratinizing Squamous Cell carcinoma	04	28.57%
Keratinizing Squamous Cell carcinoma	08	57.14%
Small Cell Carcinoma	01	07.14%
Adenocarcinoma	01	07.14%
Total	14	

When comparison of cases of acetic acid findings and biopsy was done, acetic acid positive and biopsy positive were 128, acetic acid positive and biopsy negative 230. Acetic acid negative and biopsy negative were 138, and acetic acid negative and biopsy positive were 4 (Table-6). When comparison of Pap

smear and biopsy was done; PAP Negative and biopsy negative were 360 cases, PAP Negative and biopsy Positive were 14 cases, PAP Positive and biopsy negative 08 cases, PAP positive and biopsy positive were 118 cases (Table-7).

Table-6: Comparison Of acetic Acid and Biopsy

	BIOPSY NEGATIVE	BIOPSY POSITIVE
ACETIC ACID NEGATIVE	138	004
ACETIC ACID POSITIVE	230	128

Table-7: Comparison of Results of Pap and Biopsy

	BIOPSY NEGATIVE	BIOPSY POSITIVE
PAP NEGATIVE	360	014
PAP POSITIVE	008	118
	368	132

Acetic acid and biopsy tests- Sensitivity was 96.97%, Specificity was 37.50%, Positive predictive value 48.12% and Negative Predictive value 1.71%. Similarly, Biopsy and PAP smear tests- Sensitivity was 89.39%, Specificity was 97.83 %, Positive predictive value 24.69, Negative predictive value 63.64%.

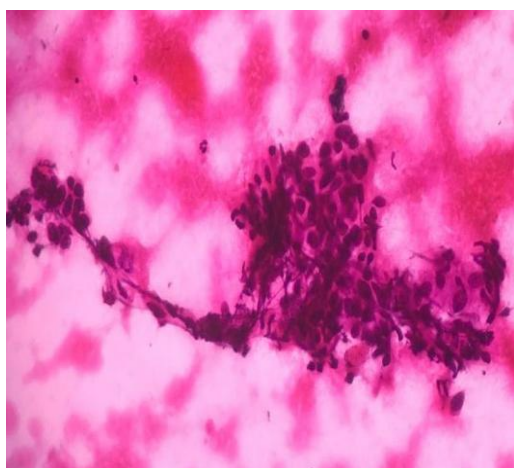


Fig-1: Cytology image showing clusters of showing enlarged cells with HSIL features

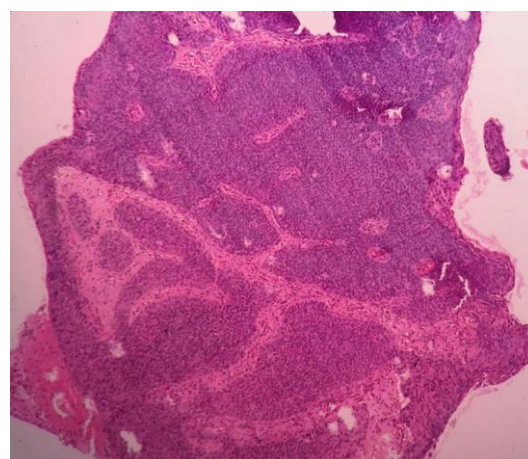


Fig-2: Histopathology image showing Carcinoma-In-Situ (CIN)

DISCUSSION

In more developed countries, pap smears have formed the basis of cervical screening and detection programs for many years. National cytology based screening programs have contributed substantially to the marked decline in deaths from cervical cancer in

these countries [5], however the technical complexity of cytological testing, and the infrastructure required to implement it, precludes the effectiveness of national Pap smear programs in India. Several reports suggested that visual inspection of the uterine cervix after the application of 3 to 5 % acetic acid can lead to satisfactory detection of cervical lesions and lesions missed by cervical cytology in less developed countries. In the present study, 500 cases of cervical lesions were studied. Women with unhealthy cervix, aged between 20 to 70 years were taken as study subjects. Maximum number of cases was seen in third decade of life (30-40 years) which is in correlation with the study conducted by Bhushan M Warpe *et al.*, [6]. Pap smears were taken and studied for all the cases. Acetic acid test was done. Biopsy was done for acetic acid positive cases. Visual inspection with acetic acid was positive in 358 cases and acetic acid negative in 142 (28.40%) cases. Pap smears were taken for all the cases. Smears were reported as normal or healthy in 126 (25.20 %) cases, rest of the 374 cases – 232 were inflammatory smears (46.4 %).

Negative for Intra-Epithelial Lesion or Malignancy (NILM) is a category under Bethesda classification of cervical cytology 2014 [121314] which includes nonspecific inflammatory pathology and infections due to organisms like trichomonas vaginalis (TV), Candida, bacterial vaginosis (BV), actinomycosis and HSV viral infection [2-4, 6].

Table-8: Shows Comparative Estimation of NilM Cases by Different Studies

VARIOUS STUDIES	PERCENT OF NILM CASES
Bhushan M. Warpe <i>et al.</i> , [6]	75.14 %
Saha R <i>et al.</i> , [7]	51.16 %
Rathore SB <i>et al.</i> , [8]	86.00%
Selhi PK <i>et al.</i> , [9]	96.08 %
Laxmi PV <i>et al.</i> , [10]	67.00%
Kalyani R <i>et al.</i> , [11]	96.92 %
Present study	46.4 %

16 cases (3.2 %) were diagnosed as ASCUS (Atypical Squamous Cells of Undetermined Significance) which is in correlation with Saha R *et al.*, Rathore SB *et al.*, Kalyani R *et al.*, and Selhi PK *et al.*, [7-9, 11]. 75 cases LSIL (15 %) and 37 cases (7.4%) HSIL were diagnosed in this study which are highest when compared to the other studies [7-9, 11]. 14 cases showed malignancy either squamous cell carcinoma or adenocarcinoma. 16 cases which were acetic acid negative were diagnosed by Pap smear as inflammatory lesions (12) and LSIL (4). Another 10 cases which were acetic acid positive were proved by Pap smear as healthy smears.

Biopsy was done for acetic acid positive cases (358 cases) or pap positive cases. Out of which 230

were chronic Cervicitis cases. 68 cases were LSIL and 44 were identified as HSIL; 2 cases as carcinoma in-situ (CIN) and 14 were carcinomas. Out of 16 pap positive cases, only 6 returned for follow up in that 4 were diagnosed by biopsy as LSIL. Out of the 14 malignancies, 4 were large cell keratinizing, 8 cases were large cell non-keratinizing. One case each of small cell carcinoma and adenocarcinoma were reported.

Results of VIA and biopsy were compared. Sensitivity of VIA was 96.97% and specificity was 37.50 %. Results of Pap and biopsy were compared, sensitivity of pap was 89.39% and specificity was 97.83 %. These results concluded that VIA was more sensitive than pap smear and pap smears were more specific.

In an Italian study involving 2400 women, colposcopy identified an atypical transformation zone in 312 women. 307 of them identified as having distinct white cervical epithelium on naked eye examination after acetic acid application [12]. Histopathological examination of biopsies from a total of 312 atypical transformation zone revealed benign lesions in 169 (54.2 %) and C1N1 or worse lesions in 143 (45.8 %). This is one of the earliest reports indicating that a cervix at risk can be identified by recognizing acetowhite areas with the naked eye.

Another Italian study involving 2105 women compared visual inspection with acetic acid, cervicography and cytology, and reported positivity rates of 25.4 %, 15.3% and 3.8% respectively, with these procedures [13]. Among 486 women with at least one positive test reported colposcopic directed biopsy was performed in 281 cases. Cytology, cervicography and VIA detected 5, 5 and 7 of the 8 CIN lesions detected from among them. VIA was found to be less specific but more sensitive than a pap smear. In another study, 85 subjects with suspicious acetowhite lesions and normal pap smears were subjected to colonoscopy. 34 (40 %) had normal colposcopic examination, and the rest were subjected to biopsy, among which 13 CIN lesions were detected.

Another study conducted in Cape Town, South Africa; involving 2426 women those positive on VIA or those with squamous intraepithelial lesions on cytology were referred for colposcopy and biopsy [14]. Of the participants in this study, 61 were positive on VIA by trained nurses plus cytology, 15 were positive on VIA only, 254 were positive for cytology only, and 2096 were negative for both VIA and cytology. Of the total of 31 histologically detected, high grade SIL lesions in the study, 20 were detected by both tests and the remaining 11 by cytology. The authors concluded that because VIA detected more than 60 % of the high grade SILs, it merited consideration as an alternative to cytology in low resource settings.

Another study conducted in Ernakulam, Kerala, India, involving 1351 women, 37.7 % were positive on VIA carried out by trained nurses and 15.2 % on cytology; 494 were subjected to colposcopy and 95 to biopsy VIA detected 95.8% of 71 biopsy proved moderate dysplasia or worse lesions as opposed to 62.0% by cytology.

The results of the current study indicate that VIA and cytology has almost the same performance in detecting cervical lesions. The proportions of women referred for biopsy are also similar. The results of the current study and other reported studies indicate that VIA is a simple and objective test. The results of this procedure are available immediately. The test is not expensive and it is possible to train providers to detect acetowhite lesions with naked eye.

CONCLUSION

Pap smears are required not only for the diagnosis and management of the malignant lesions but it is also helpful in identifying the infectious etiologies and treatment in developing countries. They need to be correlated with histopathology for further management. In developing countries, where it is not feasible to introduce pap smear screening of acceptable quality, VIA is alternative low cost method for cervical screening.

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