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Original Research Article

Comparative study of effectiveness of pap smear versus visual inspection with acetic acid for mass screening of lesions of cervix

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ABSTRACT

Background: Cervical cancer is a leading cause of morbidity and mortality among women worldwide and more over in the developing countries, so there is a need to develop screening test with high specificity and sensitivity. Aim of the study was to evaluate the effectiveness of Papanicolaou (PAP) smear versus visual inspection acetic acid (VIA) for screening cervical lesions in patients and to determine and compare their sensitivity and specificity.
Methods: The present study is a hospital based prospective study for a period of two years at the department of pathology from August 2014 to July 2016 consisting of 500 patients attending gynaecology outpatient clinic. Papanicolaou (Pap) smear tests and visual inspection acetic acid were employed along with complete clinical history record. The results of VIA were correlated with that of pap smear on the basis of sensitivity, specificity and positive and negative predictive value.
Results: Out of 500 cases, most common age group was 21 to 40 years of age consisting of 305 cases - 61%. VIA was positive in 156 cases-31.2%, PAP smear positive for epithelial cell abnormalities were 60 cases-12%. VIA showed higher sensitivity (52.38%) compared to Pap smear (40%) whereas Pap smear showed higher specificity (93.2%) compared to VIA (92.4%).
Conclusions: Papanicolaou test is a better screening test for epithelial cell abnormality than VIA. However, in countries with low resource settings where cytology-based screening programs are not available, VIA is a promising alternative.

Keywords: Acetic acid, Cervical cancer, Papanicolaou smear

INTRODUCTION

Cervical cancer is the second most common cancer in women, and 80% of these cases occur in underdeveloped countries.[1] Cervix is both a sentinel for potentially serious upper genital tract infections and a target for viruses, other carcinogens, which may lead to invasive carcinoma.[2] Cervical cytology often referred to as Papanicolaou (PAP) smear is perhaps the most well known of available screening methods. The PAP smear is a simple, safe, noninvasive, and effective method for detection of precancerous, cancerous, and noncancerous changes in the cervix and vagina.[3] Naked eye inspection of cervix with acetic acid is an alternative to cervical cytology study in screening for cervical lesions.

The use of acetic acid during visual examination of the cervix, termed visual inspection with acetic acid (VIA), has been advocated as an alternative screening method to PAP smears in developing countries.[4]

Visual inspection with acetic acid has been recommended by WHO in 1985 as an alternative to cytology to pick up a patient at risk of cancer cervix.[5] The present study is a comparative study of effectiveness of VIA with that of PAP smear for screening lesions of cervix.

METHODS

Study design

The present study is an Hospital-based prospective observational study for a period of two years at the department of pathology over a period of 24 months from August 2014 to July 2016.

Study subjects

In this study, 500 patients attending gynaecology outpatient clinic were selected. A complete history of the patient pertaining to complaints, any white discharge per vagina, postcoital bleeding, menstrual history, obstetric history and contraception history were obtained. Papanicolaou (Pap) tests and VIA were employed.

Inclusion criteria

All women with age group of 20 to 70 years with recurrent episodes of White discharge, Postcoital bleeding, Intermenstrual bleeding and Postmenopausal bleeding.

Exclusion criteria

Women with age less than 20yrs and more than 70yrs, women who refused to give consent, known cases of cervical carcinoma, pregnant women, women with active vaginal bleeding and frank invasive growth of cervix cancer.

Informed written consent was taken. Detailed clinical data were obtained and noted on a structured proforma. Per speculum examination of cervix and vagina was done. The squamocolumnar junction was visualized, with the hooked end of Ayre's spatula, squamocolumnar junction was scraped gently throughout its circumference and material was transferred to glass slides. Then endocervical brush is inserted and rotated gently throughout its circumference and material transferred to glass slides. Smears were fixed with 95% alcohol immediately and stained by Papanicolaou stain. Pap smear reporting was done according to the Bethesda classification.

After taking pap smear, the same Women were subjected to visual inspection of the cervix after application of 5% acetic acid. Using a cotton swab soaked in acetic acid was applied on cervix for one minute and then the cervix was carefully inspected for any aceto-white lesions, particularly in the transformation zone.

RESULTS

In the present study, a total of 500 cases were studied. Out of the 500 women who participated, 305 cases-61% were 21 to 40 years of age and 160 cases-32% were 4-60 years of age; the mean age was 40.84 years. Most of the cases were observed in females within active reproductive age group (Table 1).

Table 1: Age distribution (n =500).

Age	No. of cases	Percentage
0-20	4	0.8%
21-40	305	61%
41-60	160	32%
61-80	31	6.2%
>80	0	0%
Total	500	100%

Table 2: Clinical symptoms (n =500).

Clinical symptoms	Cases	%
White discharge	190	38.00%
UV prolapse	78	15.67%
Fibroid uterus	45	9.00%
AUB	33	6.67%
Cervical erosion	30	6.00%
Menorrhagia	28	5.67%
Post menopausal bleeding	27	5.4%
Ovarian cyst	18	3.67%
Pain abdomen	13	2.67%
Ovarian tumor	10	2.00%
Polymenorhagia	6	1.2%
CKD	5	1.00%
Misc	17	3.4%
Total	500	100

Table 3: VIA positive cases (n =500).

VIA	No. of cases	Percentage
Positive	156	31.2%
Negative	344	68.8%
Total	500	100%

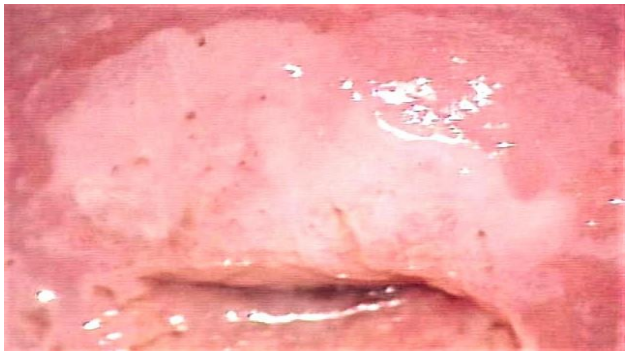

Figure 1: Visual inspection with acetic acid (VIA) showing acetowhite areas -positive.

Out of 500 cases, most common presenting clinical symptom is white discharge seen in 190 cases-38%, followed by uterine prolapse seen in 78 cases-15.67% (Table 2).

In the present study, out of 500 cases Visual inspection with acetic acid test was positive in 156 cases-31.2%. (Figure 1) (Table 3).

Out of 500 cases, Pap positive for epithelial cell abnormality cases were 60-12%, pap negative for epithelial cell abnormality- negative for intraepithelial lesions/malignancy (NILM) were 397 cases-79.4% (Figure 2) (Table 4).

Table 4: PAP smear results (n =500).

Pap smear	No. of cases	Percentage
Pap negative for epithelial cell abnormality NILM	397	79.4%
Pap positive for epithelial cell	60	12%
Abnormality	5	1%
Ascus LSIL	37	7.4%
HSIL SCC	10	2%
Glandular	2	0.4%
CELL	6	1.2%
Inadequate	43	8.6%
Total	500	100%

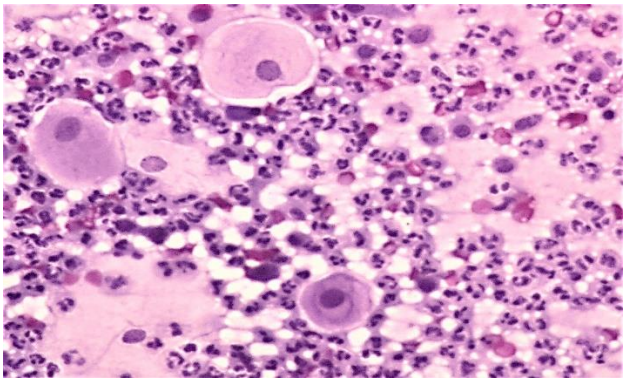

Figure 2: Photomicrograph showing squamous cells with Koilocyte change (PAP stain; 400x).

In the present study, biopsy was done in 300 cases out of 500. Out of these 300 cases, 237 cases-79% were chronic non specific cervicitis, mild dysplasia were 42 cases, moderate dysplasia were 10, severe dysplasia were 3 and squamous cell carcinoma were 8 cases (Table 5).

In the present study, out of 500 cases, VIA was positive in 156 cases-31.2%. In PAP smear tests, out of 60 epithelial cell abnormalities detected, VIA was positive in 33 cases-55% and out of 397 cases of Negative for intraepithelial lesions/malignancy (NILM), VIA was positive in 110 cases - 27.7% (Table 6).

Table 5: Biopsy results (n=300).

Biopsy results	Cases	Percentages
Biopsy negative for neoplastic lesions chronic non specific cervicitis	237	237 79%
Biopsy positive for neoplastic lesions	63	21%
Mild dysplasia	42	14%
Moderate dysplasia	10	3.3%
Severe dysplasia	3	1%
Squamous cell carcinoma	8	2.6%
Total	300	100

Table 6: PAP and VIA correlation (n=500).

Pap smear	No. of pap smears	No of VIA + (correlated)	Percentage correlated
Pap negative for epithelial cell abnormality NILM	397	110	27.7%
Pap positive for epithelial cell abnormality	60	33	55%
ASCUS	5	3	60%
LSIL	37	18	48.6%
HSIL	10	5	50%
SCC	2	2	100%
glandular cell	6	5	83.3%
Inadequate	43	13	30.3%
Total	500	156	31.2%

In the present study, out of 263 NILM cases reported on Pap, 233 cases were reported as chronic non specific cervicitis, 26 cases were (cervical intraepithelial neoplasia) CIN1 and 4 cases were CIN2. Out of 5 cases reported as Atypical Squamous Cells of Undetermined significance (ASCUS) on pap, 2 cases were reported as chronic non specific cervicitis, 2 were CIN1 and 1 case of CIN3.

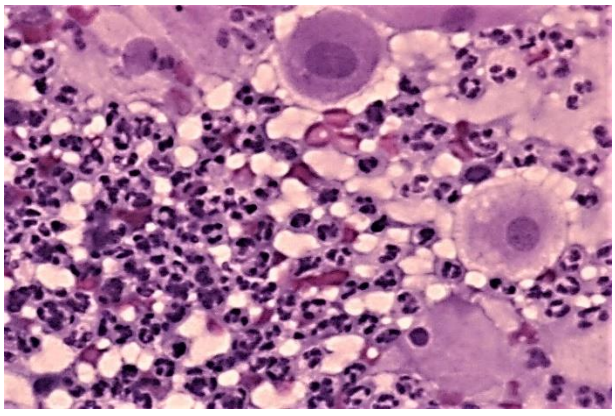

Figure 3: Photomicrograph showing cells with nucleomegaly more than half of the cell LSIL (PAP stain; 400x).

Out of 22 cases of Low Grade Squamous Intraepithelial Lesion (LSIL) (Figure 3), 2 were chronic non specific cervicitis, 14 were CIN1, 4 were CIN2 and 2 cases were CIN3. Out of 8 cases of High Grade Squamous Intraepithelial Lesion (HSIL) (Figure 4), 2 cases were reported as CIN2 and 6 cases were turned out to be squamous cell carcinoma (Figure 5) (Table 7, 8).

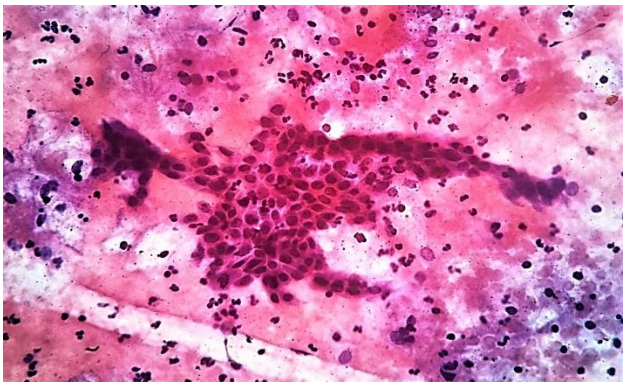

Figure 4: HSIL with marked increase in nuclear/cytoplasmic ratio, irregular nuclear membrane (PAP stain;400x).

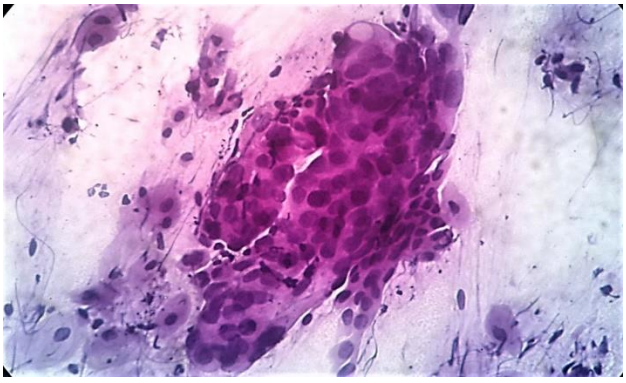

Figure 5: Sheets of malignant epithelial cells: Squamous cell carcinoma (PAP stain; 400x).

Table 7: Cyto-histopathological correlation (n=300).

	No. of Pap smears	Histopathological examination				
		Non neoplastic	CIN 1	CIN 2	CIN 3	SCC
NILM	263	233	26	4	-	-
ASCUS	5	2	2	-	1	-
LSIL	22	2	14	4	2	-
HSIL	8	-	-	2	-	6
SCC	2	-	-	-	-	2

Out of 47 deferred cases, 8 cases of LSIL on cytology, 2 cases were diagnosed as chronic non specific cervicitis,4 cases as CIN-2 and 2 cases as CIN-3 on histopathology. Out of 6 cases of HSIL on cytology, 6 cases turned out to be squamous cell carcinoma on histopathology (Table 9).

Table 8: Correlated and non correlated cases on cytohistopathology (n=300).

	No. of pap smears	Correlated	Not correlated
NILM	263	233	30
ASCUS	5	2	3
LSIL	22	14	8
HSIL	8	2	6
SCC	2	2	0

Table 9: Deferred cases of cytology and histopathology (n=47).

Cytodiagnosis	Deferred cases	Histopathology
NILM	30	CIN1-26, CIN2-4
ASCUS	3	CIN1-2, CIN3-1
LSIL	8	Ch.non.sp.cervicitis-2, CIN2-4, CIN3-2.
HSIL	6	SCC-6

Out of 300 cases for which biopsy was done, number of non neoplastic cases were 237 by histopathology, 263 by pap and 18 by VIA. Number of LSIL cases which were diagnosed on histopathology were 42, 22 by pap and 25 were positive on VIA. Number of HSIL cases which were diagnosed on histopathology were 13, 8 by pap and 3 were positive on VIA. Number of SCC cases which were diagnosed on histopathology were 8, 2 by pap and 5 were positive on VIA (Figure 6, 7) (Table 10).

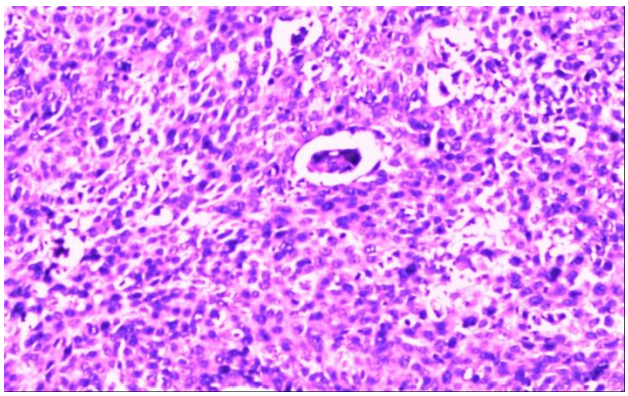

Figure 6: Non keratinizing squamous cell carcinoma. H & E stain (400x).

Table 10: VIA, cytology and histopathology correlation (n=300).

	No. of VIA positive cases	No. of PAP smears	No. of biopsy cases
Non neoplastic	18	263	237
LSIL	25	22	42
HSIL	3	8	13
SCC	5	2	8

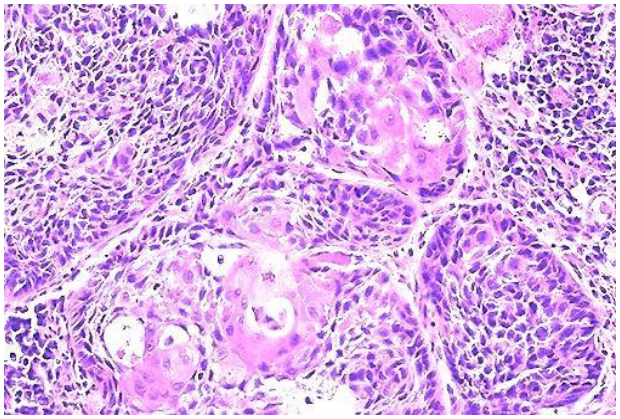

Figure 7: Keratinizing squamous cell carcinoma. H & E stain (400x).

DISCUSSION

Invasive cervical cancer is preceded by a long phase of precancerous lesion that can be detected by screening and treated effectively by simple treatment, which can prevent invasive cancer.[6] The cytological diagnosis of cervical smears has become a very important screening test for the detection of preinvasive and invasive cervical epithelial abnormalities. Screening of female population for cervical neoplasia is a simple, inexpensive and reliable method which greatly reduces the mortality and morbidity associated with carcinoma cervix, if detected in its preinvasive stage.[7]

Prompted by the need for optimal strategies for cervical cancer screening, and based upon the concepts that the majority of pre-invasive and invasive cervical lesions are visible by 'naked-eye' observation, investigators have developed novel affordable diagnostic tools suitable for large-scale screening of cervical abnormalities.[8] Several recent studies testing VIA suggest that it closely matches the Pap smear in its performance in detecting cervical cancer precursors.[9]

In the present study, out of the 500 women who participated, 305 cases - 61% were 21 to 40 years of age. Mean age was 40.8 years in the present study. Most of the cases were observed in females within active reproductive age group. Age wise distribution of cases is common among the age group of 30 to 39 years which is similar to Patil K et al, which is around the age group of 30 to 40 years and Shaheen et al where it is around 31 to 40 years.[10,11]

In the present study, most common presenting clinical symptom is white discharge seen in 190 cases-38% which is comparable to Shaheen et al, where it is 48% and Patil K et al where it is 59%.[10,11]

In the study of Bhatia R et al, Saha et al, Bhalerao A et al major presenting complaint was white discharge per vagina constituting 62.6%, 62.8%, 71% respectively.[7,12,13]

In the present study, visual inspection with acetic acid test was positive in 156 cases-31.2%, which is comparable to studies done by Shaheen et al, where it is 29.3%, and Patil K et al where It is 48% (Table 11).[11,10] Goel et al, had a rate of 12.5% of VIA.[14] The VIA-positive rate in Divya hegde et al, study was 12%.[15] Cecchini et al, reported positive VIA in 25.4% in their study, whereas Slawson et al and Megevand et al reported an incidence of abnormal VIA of 4.2 and 3.13% in their study.[16-18]

Table 11: Comparison of VIA positivity with other studies.

Similar studies	VIA Positivity in percentages
Consuls et al[19]	13.87%
Patil K et al[10]	48%
Shaheen et al[11]	29.3%
Present study	31.2%

There was a wide variation in the VIA positive rate that has been reported so far. The wide range is due to the difference in interpretation since few studies used nurses or paramedical workers to do the test. It also depends on the study population since few studies were done on a symptomatic hospital-based population and others as a mass screening test. The VIA test is also affected by the quality of acetic acid, concentration of the acetic acid, and lighting, and visualization. In the present study, a comparison was done between Pap smear and VIA with histopathology being considered as the gold standard.

In the present study, out of 500 cases, negative for intraepithelial lesions/ malignancy NILM were 397 cases-79.4%. The wide variation in the ratios of non-neoplastic to neoplastic lesions in different studies is probably due to sample size. The ratio in the present study was 6.6:1 which is comparable to study by Bhatia R et al.[7]

In the present study, 12% cases showed epithelial cell abnormality on pap smears. This percentage is almost comparible to Hegde et al where it is 11.7% and consul S et al where it is 16.2% and Shaheen et al where it is18.9%.[15,19,11] Denny reported an incidence of abnormal Pap smear as 8.2% (Table 12).[20]

Table 12: Comparison of PAP smear positivity with other studies.

Similar studies	PAP Smear Positivity in percentages
Shaheen et al[11]	18.9%
Shuchi consul et al[19]	16.2%
Hegde et al[15]	11.7%
Present Study	12%

In PAP smear tests, out of 60 epithelial cell abnormalities detected, VIA was positive in 33 cases - 55% and out of 397 cases of negative for intraepithelial

lesions/malignancy (NILM), VIA was positive in 110 cases - 27.7%. In a study done by Singh et al, out of 27 cases, 25 were detected by VIA, and only 20 cases were detected by Pap smear.[21] In a study done by Goel et al, out of 30 dysplasia cases, Pap smear was positive in only 15 and VIA in 29 cases.[14] Jeronimo et al, found that pap smear detected 15 cases out of 35 CIN 1 and 5 cases out of 13 CIN 2-3, and VIA detected 20 cases out of 35 CIN 1 and 11 out of CIN2-3.[22]

In the present study, sensitivity of VIA is 52.38% and specificity of VIA is 92.4%. Positive predictive value is 64.7% and Negative predictive value is 87.95%. In the present study sensitivity of VIA is correlating with that of Gosh P et al, where it is 52.6%and specificity of VIA is correlating with that of Basu P et al where it is 92.3% (Table 13).[23,24]

Table 13: Comparison of sensitivity and specificity of VIA.

Similar studies	Sensitivity	Specificity
Goel et al[14]	96.7%	36.4%
Gosh P et al[23]	52.6%	99%
Hegde et al[15]	70.8%	95%
Bhattacharya et al[6]	89%	87%
Basu PS and Shankarnarayan et al[24]	29.5%	92.3%
Present study	52.38%	92.4%

It was observed that there is a significant association between the histopathology report and the Pap smear report as far as the negative predictive value is concerned. VIA showed high negative predictive value of 87.95% in our study and this was similar to other studies.[14] Since VIA gives immediate result and has high negative predictive value of woman with negative VIA result can be assured immediately that she is free of disease.

In the present study Sensitivity of PAP is 40% and Specificity of PAP is 93.2%. Positive predictive value is 54.05% and Negative predictive value 88.59%.

In the present study sensitivity of PAP is correlating with that of Patil K et al and Goel et al where it is 37.68% and 50% respectively and specificity of PAP is correlating with that of Bhattacharyya et al and Patil K et al where it is 95% and 92.36% respectively (Table 14).[10,14,6]

Table 14: Comparison of sensitivity and specificity of PAP.

Similar studies	Sensitivity	Specificity
Goel et al[14]	50%	97%
Patil K et al[10]	37.68%	92.36%
Hegde et al[15]	83%	98%
Bhattacharyya et al[6]	52%	95%
Present study	40%	93.2%

In the present study, VIA showed higher sensitivity compared to Pap smear, whereas VIA had lower specificity compared to Pap smear which was similar to Bhattacharyya et al study.[6] Lower specificity of VIA when compared to Pap smear was due to the high incidence of suspected acetowhite epithelium, which might be inflammation, immature metaplasia or latent HPV infection. It was observed that there is a significant association between the histopathology report and the Pap smear report as far as the negative predictive value is concerned.

The results from present study indicate that VIA is a promising method of cervical cancer screening as it showed higher sensitivity (52.38%) compared to Pap smear (40%). Majority of the studies shown that sensitivity of VIA was more than that of Pap smear, however, Pap smear had high specificity than VIA. Our findings were similar to the pooled analysis of eleven studies done in Africa and India, on cervical cancer screening tests.[25]

The present study showed higher sensitivity of VIA, probably, because screening was performed on a group of women with symptoms, such as recurrent white discharge, abnormal uterine bleeding and suspicious look of cervix, on per speculum examination. Although introduced over a half century ago, cervical cytologic screening continues to be the most effective cancer prevention test available. Cytologic screening performed only twice in a woman's lifetime can reduce her risk for invasive cervical cancer by up to 43% and yearly screening is estimated to reduce a woman's risk by over 90%.[26]

Most of the women who undergo screening with Pap smear in developing countries do not come for follow-up or do not collect their report on time thereby leading to delay in diagnosis and treatment. The advantage of VIA is that it is a real- time screening test where results are immediately known and appropriate counselling and referral for treatment can be given. Even where cytology services are well established, VIA might be a cost-effective method of rapidly differentiating between a potentially diseased cervix and a healthy one.

CONCLUSION

At present, cytology is the standard of screening of cervical cancer. However, in countries with low resource settings where cytology-based screening programs are not available, VIA is a promising alternative. Positive cases should be subjected to biopsy to prevent unnecessary treatment. VIA specificity is comparable to Pap smear and can thus be a suitable potential alternative/adjunctive screening test not only in a resource- poor setting but in well-equipped centers also. The regimen of repeated cytological screenings and follow up of abnormal results not only increases the sensitivity but also decreases the incidence of cervical cancer.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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