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Original Research Article

Comparison of cervical acid phosphatase papanicolaou stain and standard papanicolaou stain as a screening tool for cancer cervix

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ABSTRACT

Background: Carcinoma cervix is the second most common cancer in women (18%). Cervical smear by routine Papanicolaou (Pap) smear screening has significant rates of false-positive and false-negative results. To minimise this, we compare the efficacy of cervical acid phosphatase-papanicolaou (CAP-Pap) smear with conventional Pap smear in detecting premalignant lesions of cervix. The red-colored granules (enzyme coated abnormal cells) that are clearly detected, CAP-Pap positive aids in the quick and early diagnosis of aberrant cells, which speeds up the screening procedure

Methods: The present observational study conducted among the patients presenting to gynaecology department for cancer cervix screening. Two cervical smears were collected for Pap and CAP-Pap staining. Cytology report showing abnormality either in Pap smear or CAP-Pap smear were subjected for colposcopy guided cervical biopsy after VIA/VILI and their results were correlated with histopathology reports as gold standard.

Results: Total of 321 women were enrolled in the study. On comparison with Cervical biopsy, Pap smear had a sensitivity of 83.3%, specificity of 50%, Positive predictive value of 88.2%, Negative predictive value of 40.0%. CAP-Pap had a sensitivity of 100%, specificity of 50%, PPV of 90%, NPV of 100%. As evidenced by the 100% sensitivity, CAP-Pap meets the screening test criteria.

Conclusions: The CAP- Pap test has a bright future as a rapid, inexpensive, and efficient method for initial screening or as an addition to Pap smear in primary health care of India for effective cervical cancer screening.

Keywords: Papanicolaou, Cervical acid phosphatase test, Premalignant, Biopsy, Screening

INTRODUCTION

Carcinoma of cervix accounts for 15% of all cancers diagnosed worldwide and is the second most common cancer in women. In India in 2018 it was estimated that approximately 96,922 women develop cervical cancer and almost 60,078 died due to cancer cervix.[1] Persistent infections with one of 15 oncogenic strains of human papillomavirus (HPV) cause cervical carcinomas. Cervical pre-invasive lesions develop to invasive carcinomas over a long period of time. As a result, most lesions may be detected early by screening programs before they progress to malignancy. Only persistent HPV infections of which 1% of LSIL and 5-12% of HSIL develop into invasive lesions. Cancer cervix occupies either the top rank or second among cancers in women in developing countries, whereas in the developed countries cancer cervix does not find a place even in top five leading cancers in women. This is due to routine cancer cervix screening. Cervical smear cytology screening by Papanicolaou (Pap) smear is the cost-effective method of cancer screening, decreasing the incidence and -mortality

from cervical cancer. However, cervical smear screening has significant rates of false-positive and false-negative results, ranging from 10.3% for false positive cases to 5.6% for false negative cases.[2,3,4] To improve the detection and screening of precancerous lesions of the cervix a HPV DNA tests are available which are expensive and can be done only in a tertiary center. To over-come these problems a cost effective cytochemical stain was introduced to measure the acid phosphatase activity in the cervical epithelium.[5] Since the description of the new cervical acid phosphatase test (CAP Test) for visualization of cervical acid phosphatase activity (CAP) inside abnormal cervical cells on smears, it has become possible to explore this enzyme as a biomarker for cervical dysplasia, and as a possible surrogate for PAP smear in detection of cervical intraepithelial neoplasia (CIN).

Rationale and novelty

Rationale of current study was to identify efficacy of screening test in detecting preinvasive and invasive lesions of cervix to increase the sensitivity and specificity of routine PAP test. Novelty in current study was CAP PAP acts as a quick and efficient method. The test promises a great future in health centers as the paramedical staff can be easily trained for identification of abnormal cells.

METHODS

Study design

Current investigation was a hospital based observational study. Patients presenting to Gynaecology OPD of AVMCH from November 2019 to November 2021 full filling inclusion and exclusion criteria were included in study.

Inclusion and exclusion criteria

Inclusion criteria for current study were; 3 years after marriage, sexually active women>21 years, H/o post coital bleeding, High risk factors for carcinoma cervix, high risk behavior in the subject or husband partner of the subject, postmenopausal bleeding, abnormal VIA/VILI test. Exclusion criteria of current study were; pregnancy, menstruating woman., overt growth in cervix, previous treatment of cancer lesion of cervix, cervix showing obvious inflammation, woman <21 years of age.

Sample size calculation

Sample size was estimated by using based on the sensitivity and specificity of PAP at 75% and 100% with respect to histopathology findings obtained from the study Batra et al. Considering 10% non-response rate, sample size of 300 cases are to be reported. Cervical histopathology will be included in the study. Sampling technique was consecutive sampling.

Study procedures

Total 321 females undergoing cervical cancer screening at OBG department of AVMC, demographic details, routine gynaecological history and physical examination was noted. After obtaining informed and written consent were studied fulfilling both inclusion and exclusion criteria, In the absence of bleeding, obvious infection OR growth Two cervical smears were collected, for PAP and CAP-PAP staining. Cytology report showing abnormality either in PAP smear or CPAP smear are subjected for colposcopy guided cervical biopsy after VIA/VILI and their results were correlated with histopathology reports as gold standard. Comparing the sensitivity, specificity, PPV, NPV for both PAP and CPAP. Independent variables were; age, parity, socioeconomic status, co-morbities. Outcome variables were; abnormal squamous cell (pre invasive lesions of cervix). Benefits of current study were; in screening of cervical cancer on a large-scale CAP-PAP acts as a quick and efficient method, test promises a great future in the health centres as the technicians can be easily trained for identification of abnormal cells

Statistical analysis

All the data was entered in excel sheet and analysed using SPSS v21 operating on windows 10. The demographic details of the patients were summarized as frequency, percentage, mean, standard deviation and represented as table, figure, bar diagram and pie chart. The difference between categorical data was analysed using chi-square test and the diagnostic accuracy, sensitivity, specificity, PPV and NPV was analysed. A p value of <0.05 was considered to be statistically significant.

RESULTS

Total of 321 women were enrolled in present study after obtaining the informed consent. The mean age of participants included in the study was 44.97 yrs of age.

Table 1: Mean age and chief complaints of the patients.

Parameters	Mean	SD
Age (years)	44.97	12.53
Chief complaints	**N**	**%**
Discharge PV	74	23.1
PMB	10	3.1
Post coital bleeding	4	1.2
Routine screening	233	72.6
Total	321	100.0

Chief complaints were found to be discharge PV in 23.1% of women, 3.1% with PMB, 1.2% with post coital bleeding and 72.6% were on routine screening process. On assessment PAP smear was positive in 17 patients and 305 were negative for the premalignant lesions. Among the included patients, 8.7% were presented with atrophic

changes, 5.6% with bacterial vaginosis, 3.7% with inflammatory changes, and 2.5% with Candidiasis. Other minor changes included the trichomonas vaginalis and unsatisfactory result. On assessment of the premalignant changes, 0.3% presented with ASCUS, 2.5% LSIL, 0.3% with ASC-H, 0.9% with HSIL, 0.6% with SCC. Among 321 patients screened 20 were positive for malignancy by CPAP were subjected to cervix biopsy.

Table 2: Results on PAP smear assessment.

Parameters		N	%
PAP results	NILM	230	71.7
	Inflammatory smear	14	4.5
	Bacterial vaginosis	18	5.6
	Candidiasis	8	2.5
	Trichomonas vaginalis	3	0.9
	Atrophic	28	8.7
	ASCUS	3	0.9
	LSIL	8	2.5
	ASC-H	1	0.3
	HSIL	3	0.9
	SCC	2	0.6
	Unsatisfactory	3	0
	Total	321	100.0

Table 3: CPAP result of the included patients.

Parameters		N	%
CPAP	Negative	301	93.8
	Positive	20	6.2
	Total	321	100.0

Table 4: Cervix biopsy results of the included patient.

Parameters		N	%
	Not done	299	93.1
Cervix biopsy	Negative	04	1.3
	HSIL	04	1.3
	LSIL	11	3.4
	SCC	03	0.9
	Total	321	100.0

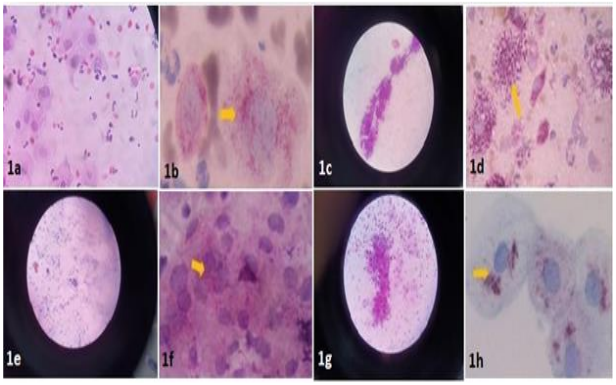

Figure 1: a, b) LSIL and CPAP LSIL; c, d) HSIL and CPAP HSIL; e, f) ASC-H and CPAP ASC-H; g, h) ASCUS and CPAP ASCUS.

Among the 22 patients who were positive either by PAP or CPAP were subjected to cervix biopsy and their results were 4 were negative for malignancy. Among the remaining 18 patients 4 were HSIL, 11 were LSIL, 3 were SCC. For 17 PAP smear cases biopsy showed positive for 15 patients (2 false positive), similarly for 20 CPAP positive patients biopsy showed positive for 18 (2 false positive). For 3 inflamatory smears which was positive in CPAP turned to be premalignant in biopsy. On assessment of CPAP results with cervix biopsy we found a sensitivity of 100%, specificity of 50%, PPV of 90%, NPV of 100%. On comparison of the PAP results with the cervix biopsy, we found a sensitivity of 83.3%, specificity of 50%, PPV of 88.2%, NPV of 40.0%.

DISCUSSION

The present observational study conducted among the patients presenting to department of gynaecology AVMCH fulfilling the inclusion criteria. Aimed to determine the sensitivity and specificity positive predictive value and negative predictive value of Acid phosphatase Papanicolaou smear in comparison with conventional pap smear in detecting premalignant lesions of cervix. Total of 321 women were enrolled in the present study after obtaining informed consent. The mean age of participants included in the study was 44.97±12.53yrs of age, which was in concordant with Nkwabong whose mean age was 43.5±10.5. In this study among the included study participants, majority were in the socioeconomic class IV (59.2%), followed by class III SES (38.9%). In this study among study participants the chief complaints were found to be discharge PV in 23.1% of women, 3.1% with PMB, 4% with post coital bleeding and 72.6 are for routine screening which is concordant with Rajini et al study whose major complaint among the study participants were discharge PV. In our study among participants 11.2% of women had type 2 diabetes mellitus, 6.5% with systemic hypertension and 6.2% anaemia. In our study on assessment of PAP smear, were found to be positive in 17 patients and 305 were negative for the premalignant lesions. Among the included patients, 8.7% were presented with atrophic changes, 5.6% with bacterial vaginosis, 4.5% with inflammatory changes, and 2.5% with candidiasis. Other minor changes included the trichomonas vaginalis and unsatisfactory results. On assessment of the premalignant changes, 2.5% presented with LSIL, 0.9% with HSIL, 0.6% with SCC and 0.3% with ASC-H and 0.9% ASCUS was in concordant with the study by Deb et al., On routine reporting, out of a total of 81 smears, 73 were reported ‘negative for intraepithelial lesion or malignancy’, which included one case each of an atrophic smear, candidiasis, and Trichomonas vaginalis. Three smears were unsatisfactory. Of the remaining smears, one was LSIL and four were atypical squamous cells of undetermined significance (ASCUS), of which one was combined with atypical glandular cells of undetermined significance.[5] For all 17 positive PAP smear cases, cervix biopsy was taken and it was positive for 15, suggesting that in PAP

smear there are 2 false positive results. Similarly with 20 CPAP positive cases cervix biopsy was taken and it showed 18 cervix biopsy positive results, 2 false positive results noted.

Table 5: Comparison of PAP smear, CPAP smear and cervical biopsy results in all CPAP positive cases.

Parameters	PAP smear	CAP positive	Cervical Biopsy			
			Negative	LSIL	HSIL	SCC
NILM	2	2	2	-	-	-
Inflammatory	3	3	-	2	1	-
ASCUS	3	1	2	1	-	-
LSIL	8	8	-	8	-	-
ASC-H	1	1	-	-	-	1
HSIL	3	3	-	-	3	-
SCC	2	2	-	-	-	2

Table 6: Comparison of the findings of cervix biopsy with CPAP result using Chi-square test.

Parameters		Cx Biopsy		Chi-square (p value)
		Negative	Positive	
CPAP	Negative (but PAP positive)	02	0	321.0 (0.001)
	Positive	02	18	

Table 7: Diagnostic characteristics of CPAP smear with cervix biopsy.

Statistic	Value (%)	95% CI
Sensitivity	100.00	79.41% to 100.00%
Specificity	50.00	98.80% to 100.00%
Positive predictive value	90.00	-
Negative predictive value	100.00	-

Table 8: Comparison of the findings of cervix biopsy with PAP result using Chi-square test.

Parameters		Cx Biopsy		Chi-square (p value)
		Negative	Positive	
PAP	Negative (but CAP positive)	02	3	299.95 (0.001)
	Positive	02	15	

Table 9: Diagnostic characteristics of PAP smear with cervix biopsy.

Statistic	Value (%)	95% CI
Sensitivity	83.3	69.77% to 99.84%
Specificity	50.00	98.80% to 100.00%
Positive predictive value	88.2	-
Negative predictive value	40.0	97.86% to 99.95%

On combining PAP and CPAP positive results there were 17 cases for whom biopsy showed positive for all 17 cases. In this study cervix biopsy was done for 22 patients (for PAP positive and CPAP positive patients) comprising 6.9% overall participants, which showed HSIL 1.3%, LSIL 3.4%, SCC 0.9%. In this study out of 321 enrolled patients 20 were positive for CPAP, on comparing their results with PAP smear and cervix biopsy we found 2 CPAP positive smears which showed NILM in PAP smear was negative for malignancy in biopsy. Also 3 CPAP positive results which was shown as inflammatory smear in PAP was found positive for malignancy in cervical biopsy. 3 PAP smear showed ASCUS in results for whom CPAP was positive for only 1 patient which was confirmed by cervical biopsy as premalignant lesion, remaining 2 ASCUS (PAP) smears were negative for both CPAP and cervix biopsy. In this study on comparison of the PAP results with the cervix biopsy, we found a sensitivity of 83.3%, specificity of 50%, PPV of 88.2%, NPV of 40.0%. Similarly on assessment of CPAP results with cervix biopsy we found a sensitivity of 100%, specificity of 50%, PPV of 90%, NPV of 100%. On assessment of two methods, PAP and CPAP was equally efficient in detecting premalignant and malignant lesions of cervix, but when both the test are combined for screening sensitivity and specify, positive predictive value and negative predictive value can be increased which was similar to present study,

Batra et al found the sensitivity on PAP smear was 75% and specificity of 100% and CPAP smear was found to be 100% sensitive and 97.2% specific. In study by Deb et al when compared to the standard Pap stain, the CAP-PAP screening had a sensitivity of 100 percent and a specificity of 89 percent. A positive CAP-PAP test had a 50% predictive value, whereas a negative test had a 100% predictive value. In the research, there were no false negatives, however there were 11 percent false positives which was in concordant with our study.[5] A variety of histochemical reactions have been studied in benign and malignant uterine cervix lesions, including ribonucleic acid, glycogen, acid phosphatase, nonspecific esterase, glucuronidase, and phosphamidase. Acid phosphatase drew the most attention because of its ease and intracellular localization utilising cytochemical methods.[6-11] As evidenced by the 100% sensitivity, CPAP meets the screening test criteria, hence this test might be highly beneficial as a rapid, inexpensive, and efficient means of wide scale screening. Also on combining both PAP and CPAP the specificity of test can be increased. Because of the red-coloured granules (enzyme coated abnormal cells) that are clearly detected, CPAP positive aids in the quick and early diagnosis of aberrant cells, which speeds up the screening procedure. The test has a bright future in basic health care settings in India, where trained personnel are scarce; paramedical staff may be quickly trained to identify abnormal smears.

Limitations

As the prevalence of precancerous lesion is more in INDIA, sensitivity and specificity can be increased by doing study in community based population. Since the sample size was small adequate positive results were not obtained.

CONCLUSION

The CPAP test may be used primarily for initial screening or as an addition to PAP smear to increase the specificity of cervical cancer. Furthermore, this approach looks promise in low-resource situations where skilled cytologists are unavailable. When used in combination with routine Pap smears, it has the potential to increase specificity, resulting in a more effective cervical smear-screening programme. Cervical acid phosphatase enzyme is very useful in picking up abnormal cells even in inflammatory smear.

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

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