# ORIGINAL ARTICLE

# CERVICAL ACID PHOSPHATASE: EVALUATION AS AN ADJUVANT TO PAPANICOLAOU SMEAR SCREENING IN CERVICAL CANCER DETECTION

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**ABSTRACT: INTRODUCTION:** Carcinoma of cervix accounts for 15% of all cancers diagnosed worldwide and is the second most common cancer in women. In the year 2000 there were over 4,71,000 new cases diagnosed and 2,88,000 deaths from cervical cancer.(1) Approximately 79% of these deaths occurred in developing countries.(2) Cervical cancer is preventable, but most women in poorer countries do not have access to effective screening programs. In India it is estimated that approximately 100,000 women develop cervical cancer each year.(3) 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 screening by cervical smear. Cervical smear cytology screening by Papanicolaou (Pap) stained smears is the most efficacious and cost-effective method of cancer screening, decreasing the incidence and mortality from cervical cancer.(4) 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.(5,6) To improve the detection and screening of cancerous and precancerous lesions of the cervix a number of sophisticated tests are available which are expensive and can be done only in a tertiary laboratory. To over-come this problems a cost effective cytochemical stain was introduced to measure the acid phosphatase activity in the cervical epithelium.(7) 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). **AIMS AND OBJECTIVES:** To assess the utility of Cervical Acid Phosphatase stain as an adjuvant to Papanicolaou smear. **BACKGROUND:** Pap test is the most used and probably the most successful and economical cancer prevention measure currently available. It is recommended for prophylaxis of women.(8) The staining procedure was introduced by George Papanicolaou in the years 1940.(9) This procedure dramatically improved detection of cervical cancer in situ and, more important, cervical dysplasia. Both conditions were followed by aggressive treatment including surgery. As a result many lives were saved.(10) Pap test screening of healthy or oligosymptomatic women resulted in sharp reduction of cervical cancer incidence and mortality rates. Reported are reductions of 80% (Iceland), 70% (U.S.), 50% (Finland) and 34% (Sweden). The major obstacle for reaching this ultimate goal of every disease prevention is the high rate of false negative readings of the Pap test, during the primary screening. False negative rates in various literatures ranging from 1.1 to 69% have been reported.(11) Sampling and technical error, are under thorough investigation, and much effort has been given to improve

techniques.[8] Also false positive cases were reported, with rates ranging from 10.3% to14.8%.[5] To minimize these a new histochemical stain is compared with PAP to being down the false negative and false positive cases.

**KEYWORDS:** Cervical acid phosphatase (CAP), ASCUS, LSIL, HSIL, PAP, carcinoma cervix.

**INTRODUCTION TO CAP:** Acid phosphatase is very common enzymes found in plants and animals. In humans they have been intensely investigated in prostate, liver, kidney and connective tissue, particularly blood cells.[12,13] As enzymes they shown species and tissue specificity.

Medical literature contains only a few articles related to cervical acid phosphatase. In 1960, Gross and Kinzie found the gradient of acid phosphatase activity in malignant epithelium malignant cells had a higher degree of activity. In 1961 Berger showed semi-quantitative difference between acid phosphatase activity in basal and malignant cells. Mature cervical epithelial cells did not present that type of activity.[14] In 1974, Malvi et al described acid phosphatase in carcinoma of the cervix uteri. Using a staining technique according to Gomori, they found increased enzyme activity in malignant cells as to normal activity in basal cells.[14, 15]

By using an azo dye diazonium salt technique it was observed that demonstration of acid phosphatase activity inside abnormal cervical cells could be demonstrated.[16,17,18]

Many human cell types and tissues contain acid phosphatase. In humans, acid phosphatase is confined inside lysosomes.

**PRINCIPLE OF THE CAP TEST:** The intracellular chemical reaction involving acid phosphatase splitting a naphthol substrate and donating the aromatic ring to a diazonium salt producing an insoluble colourful deposit that precipitates inside cytoplasm at sites of enzyme activity. The remaining aromatic moiety of the molecule simultaneously couples with Fast Garnet GBC producing an insoluble brown-red diazonium salt on the sites of the enzyme activity. CAP activity appears as a distinct red-brown granular deposit. Counterstaining of nuclei is done by haematoxylin.

Sigma procedure for staining leukocyte acid phosphatase Cat. No. 387 used for staining peripheral blood smears, bone marrow preparations and other cytological smears were used for this test.[19]

**MATERIALS AND METHODS:**

a) **Source of data**: Patients attending OBG out-patient department at Vydehi institute of medical sciences and research, who are undergoing PAP cervical screening form the source of material. It was a duration based study of cases attending OBG OPD from 01-06-2008 to 31-05-2009.
b) **Method of collection of data**: Healthy female patients attending OBG Dept for routine cervical screening were examined by the gynaecologists, who took 2 PAP smears, one was fixed in alcohol/ commercially available spray fixative (Cytofix); the other was air dried and the smears were sent to the cytology department along with requisition form and a informed consent was taken from the patients.

**FIXATION:** Unfixed air dried smears can be kept at room temperature for several days without appreciable change in CAP activity.

Fixation of the "CAP" slide is fixed using a fixative provided by the kit manufacturer.[18]

**STAINING:** Was done as per the manufacturer's instructions.

Counterstaining by Haematoxylin was done, air-dry and mounted with DPX.

**PAP STAINING:** The "PAP" slide was stained with the rapid Pap staining kit using rapid kit, following manufactures instructions and procedure (pap staining kit was used).

The "PAP" smear is evaluated by The Bethesda 2001 classification.[20]

**CAP EVALUATION:** The "CAP" smear is evaluated for presence and degree of acid phosphatase activity among cervical epithelial cells. Cytochemical criteria are used for assessment of enzyme activity as listed in Table 2.

Roughly 100 cells are screened and the degree of each is added and cells/smear classified according to the activity as given in table 1.

The internal control for all the slides are noted (monocytes, macrophages, histiocytes) and only if the internal control is positive the slides are considered for evaluation.

**RESULTS:** On routine PAP smears screening the mean age of presentation in our study was 40.40% as compared to 35.4% in a study done by Prabal Deb et al.[7]

In our study, out of a total of 146 smears 139 (95.21%) were reported 'Negative for Intraepithelial Lesion or Malignancy (NILOM) which included as in table 6.

95.21% of the slides were negative for intraepithelial lesion or malignancy in our study as compared to 90.12% in a study done by Prabal Deb et al[7] and 73.8% in study done by Sherwani R K et al.[21]

In a study done by Kaustube Mulay et al,[22] only 1.39% of the study population had positive PAP as compared to 4.78% in our study.Other studies showed 5.9% in Lucknow, and 1.87% in a study done in Gujarat done by Mishra JS et al.[23] Among the PAP positive, 3.42 % were LSIL, 0.68% were HSIL, and 0.68 were ASCUS as compared to 0.21% for LSIL, 0.16% for HSIL, and 0.64% for ASCUS in a study done by Kaustube Mulay et al.[22] On re-evaluation of routine PAP smears with CAP smears, among the 139(95.21%) routine smears initially considered negative, 1 was re-evaluated as LSIL while another was re-evaluated as ASCUS. In each of these instances, strong CAP-positive, large squamous cells were identified in CAP-PAP smears. Careful searching of the corresponding Pap smears located a few cells, evaluated and finally diagnosed as LSIL and ASCUS. In our study we found that the slide positive rate increased on doing CAP as compared to PAP from 4.79% to 6.16% similar results were obtained by O Markovic et al[24] from 0.049% to 0.157% in another study[25] there was doubling of positive slides from13% to 27%. In another study Markovic O et al[17] 16.6% of the slides were positive in CAP as compared to 8.2% in PAP. In order to find out if there was difference in CAP and PAP smears examined, we tested the agreement between the two. We found that the observed agreement was 97% and the expected agreement was 36%. Statistical significance was assessed by applying Kappa test and

was found to be 97%, which is more than 80%, therefore we found that there was considerably good agreement between PAP and CAP.

However we calculated the increase in yield by combining CAP and PAP tests. There was a considerable increase in yield from 4.75% with PAP alone to 6.85% with CAP and PAP combined. Similar observation has been done by Prabal Deb et al[7] where there was an increase in yield from 6.17% to 19.75 %. Thus it can be clearly suggested that in spite of good agreement between PAP and CAP, CAP acts as a valuable adjuvant to PAP test in screening of cervical smears.

Our study showed high prevalence of abnormal PAP findings in the age group 51 years to 60 years as compared to a study done by Sharwani RK et al[21] it was found that the maximum abnormal PAP were in the age group 21 to 40 years(Table 3).

On testing the sensitivity of PAP alone with that of CAP and PAP combined, PAP was only 70% sensitive in detecting dysplastic lesions. Similar results were obtained by Markovic N[24] who showed an increase in sensitivity from 51.3% to 83.7% between PAP alone and CAP/PAP combined.

**DISCUSSION:** The CAP-PAP stain was easy to perform following the manufacturer's instructions and gave uniform staining results.[19] Endocervical cells always showed marked cytoplasmic granular red deposits. There was no extra cellular diffusion although in many instances, the positivity was intense and obscured nuclear details. Squamous metaplastic cells were always CAP-positive. Red granules were seen mostly as small foci diffusely distributed in the cytoplasm of metaplastic cells, although many cells showed quite intense staining of most of the cytoplasm. There was no deposition on the nucleus whose morphology could be evaluated.

Parabasal, intermediate, and superficial cells were uniformly negative with some cells showing faint granules, except in smears considered positive with CAP. Of the inflammatory cells monocytes and histiocytes showed positivity and showed most intense cellular reaction with CAP. Smears having CAP-positive mature squamous cells or CAP-positive squamous cells with nuclear enlargement or nuclear atypia were considered positive in the CAP test. In such smears, CAP-positive cells were seen focally, either as isolated cells or cell groups. Staining intensity was variable but usually quite intense and unmistakable. Smears showed highly pleomorphic cell with pleomorphic nuclei and dense CAP positivity, the staining occupied whole of the cytoplasm and obscured the nuclear features (HSIL). While in some smears, metaplastic cells which showed CAP positivity were not considered in the positive sample. No abnormal cells were identified on the corresponding routine Pap smears of these cases. The remaining 137 smears were negative for CAP. Of 9 cases found to be positive by CAP, 7 were initially positive in routine Pap smears (considering ASCUS and above as positive). After the CAP test, 2 more smears were upgraded to being positive. In one instance, squamous cells with LSIL had been overlooked due to inflammatory back ground, while in one instance; focal atypical cells with features pointing toward the suspicion of ASCUS were identified.

**LIMITATIONS:** Although technical simplicity and ability to detect SIL lesions appears to be advantageous for the CAP test, this technique does have many interpretation problems. In

comparison to the conventional Pap stain, the modification adopted for this test appears to be technically inferior in evaluating nuclear features as nuclear features are lost by air drying. Nuclear details are the most essential criteria for detecting atypical squamous cells. The presence of enzyme activity in squamous metaplastic cells implies that only a person well-versed with routine cytopathology can evaluate a CAP smear.

**RECOMMENDATIONS:** The role of the CAP test in screening for cervical cancer needs greater evaluation. A screening technique requires high sensitivity. The CAP test fulfils this criterion. We feel that its greatest utility would be for quality assurance and selecting cervical smears for re-screening. CAP positivity helps to focus attention on only the significant cells. Ignoring the CAP-negative cells greatly speeds up the screening process. All patients with CAP-positive smears can be re-called for re-evaluation.

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| Sl. No | Dysplasia (Cytological) | CIN | The Bethesda System | CAP Score |
|---|---|---|---|---|
| 1 | Benign | Benign | Normal | <100 |
| 2 | Benign with inflammation | Benign with inflammation | Normal-Benign-Infection-Reactive-ASCUS/AGCUS | <100 |
| 3 | Mild dysplasia | | Low grade SIL | >100 |
| 3 | Moderate Dysplasia | CIN I | ASCUS/AGCUS Low grade SIL | >150 |
| 3 | Severe Dysplasia | CIN II | High grade SIL | >150 |
| 4 | Carcinoma In Situ | CIN III | High grade SIL | >150 |
| 5 | Invasive Cancer | Invasive cancer | High grade SIL | >200 |

Table 1: Classification Systems for cervical smears

| CAP Activity | Degree | Visible characteristics |
|---|---|---|
| Per Cell | 0=Negative | No visible granules |
| | 1 = Low | Few granules, barely visible |
| | 2 = Moderate | Several to many granules, clearly visible, scattered through cytoplasm |
| | 3 = High | Abundance of granules, large granules, aggregates |
| Per Smear | 0 = Negative | Majority of cells negative; some cells with low activity |
| | + = Nonnegative | All degrees of positivity (Majority of cervical cells (squamous, parabasal and basal) present some degree of activity (low or moderate). One or two squamous cells or clusters of cells with high activity. Majority of cells with moderate or high activity. Atypical cell(s) with any degree of activity) |
| | | |
| Internal Control of staining | 0 = Negative<br>+ = Positive | Monocytes – histiocytes Repeat staining<br>Monocytes – histiocytes Accept results |

Table 2: Criteria for screening of CAP stained smear

| PAP Diagnosis | Number | Percentage (%) |
|---|---|---|
| NILOM | 139 | 95.21 |
| ASCUS | 1 | 0.68 |
| LSIL | 5 | 3.42 |
| HSIL | 1 | 0.69 |
| **Total** | **146** | **100.00** |

Table 3: PAP smear findings among study subjects

| CAP Diagnosis | Number | Percentage (%) |
|---|---|---|
| NILOM | 137 | 93.84 |
| ASCUS | 2 | 1.37 |
| LSIL | 6 | 4.11 |
| HSIL | 1 | 0.68 |
| **Total** | **146** | **100.00** |

Table 4: CAP Smear Findings Among the study subjects

| | PAP Positive | PAP negative | Total |
|---|---|---|---|
| CAP Positive | 6 | 3 | **9** |
| CAP Negative | 1 | 136 | **137** |
| **Total** | **7** | **139** | **146** |

Table 5: Assessment of Agreement between PAP and CAP

| | CAP Positive | CAP Negative | Total |
|---|---|---|---|
| PAP Positive | 0.4 | 6.6 | 7 |
| PAP Negative | 8.6 | 130.4 | 139 |
| **Total** | **9** | **137** | **146** |

Table 6: Expected Agreement between CAP and PAP tests

| | Combined CAP&PAP Positive | Common |
|---|---|---|
| Only CAP positive | 9 | 6 |
| Only PAP positive | 7 | 6 |

Table 7: Assessment of yield when both CAP and PAP tests are combined

| Age group | Number | PAP/CAP Positive | Percentage (%) |
|---|---|---|---|
| 20-30 | 29 | - | 0 |
| 31-40 | 45 | 3 | 2.05 |
| 41-50 | 46 | 3 | 2.05 |
| 51-60 | 26 | 4 | 2.74 |
| **Total** | **146** | **10** | **6.85** |

Table 8: Age wise disease distribution in both CAP and PAP smears

| | CAP | | PAP (%) |
|---|---|---|---|
| | **Number** | **Percentage (%)** | **Number (%)** |
| Positive findings | 1 | 2.22 | 0(0) |
| Negative findings | 44 | 97.78 | 45(100) |
| **Total** | **45** | **100.00** | **45(100)** |

Table 9: Detection of abnormal lesions among asymptomatic women

| | CAP | | PAP |
|---|---|---|---|
| | **Number** | **Percentage (%)** | **Number** |
| Positive findings | 3 | 3.65 | 2 |
| Negative findings | 79 | 96.35 | 80 |
| **Total** | **82** | **100** | **82** |

Table 10: Detection of abnormal lesion among normal per speculum examination

| | CAP/PAP combined | | |
|---|---|---|---|
| | **Positive** | **Negative** | **Total** |
| PAP Positive | 7 | 0 | **7** |
| PAP Negative | 3 | 136 | **139** |
| **Total** | **10** | **136** | **146** |

Table 11: Comparison of PAP test alone with combined CAP/PAP Result

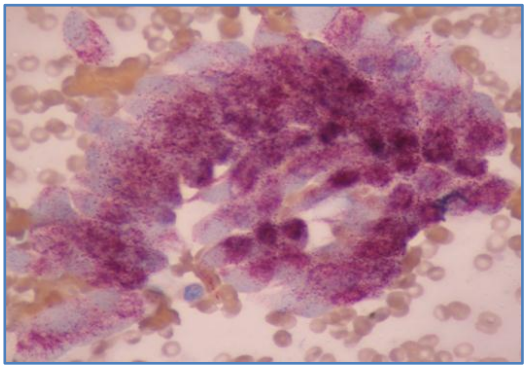

**Fig. 1: CAP Positive (3+) Endocervical Cells CAP stain 40X**

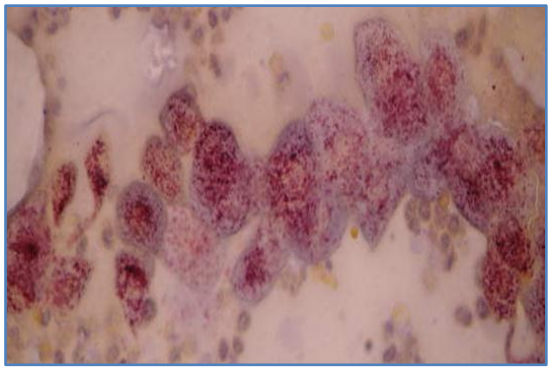

**Fig. 2: Metaplastic Squamous cells (2+). CAP stains 40X**

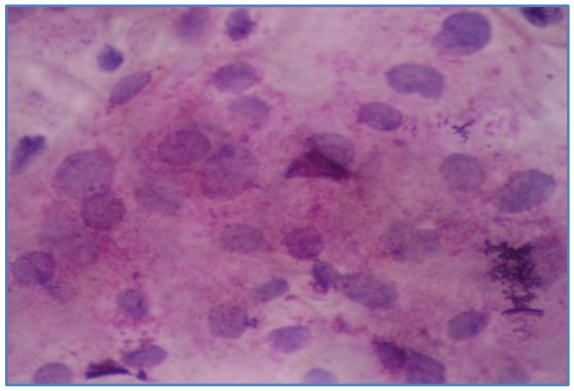

**Fig. 3: LSIL, CAP Positive (2+) cells CAP stain 40X**

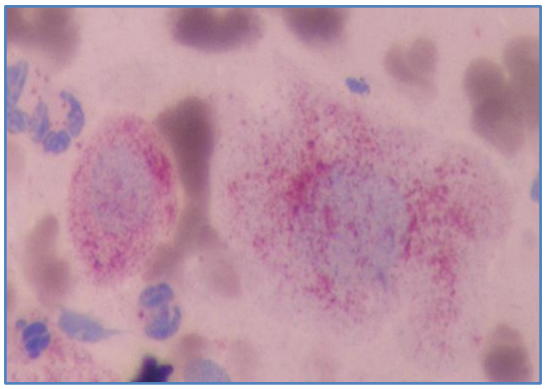

**Fig. 4: LSIL, CAP (2+) Positive cell, CAP stain 40X**

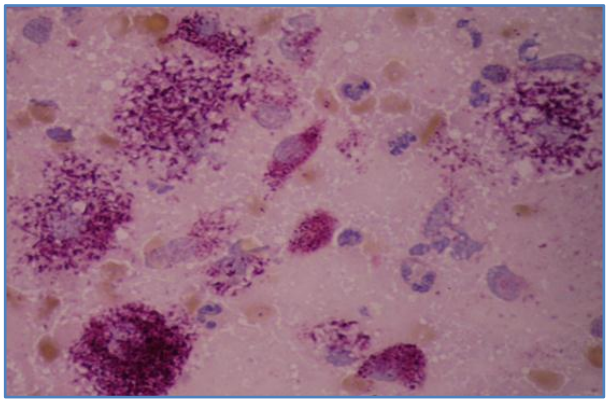

**Fig. 5: HSIL CAP (3+), CAP stain 40X**

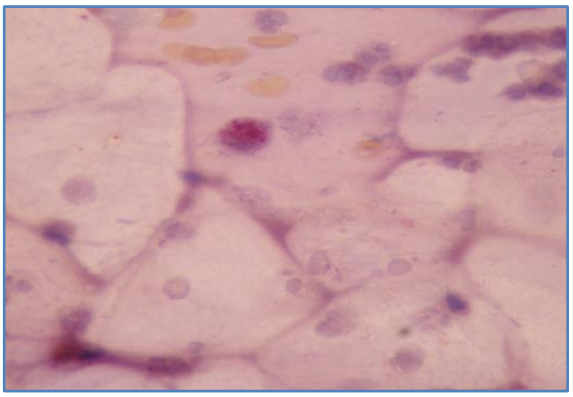

**Fig. 6: Positive internal controls (monocyte) & Negative superficial cells, CAP stain 40X**

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