International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

*Original Research Article.*

# Diagnostic Utility of Immunocytochemistry in Serous Effusions

**Shilpa Dash[1], Gayatri Rath[2*], Lity Mohanty[3]**

**[1]PG Student, [2]Assistant Professor,**
**Department of Pathology, S. C. B. Medical College, Cuttack, Odisha, India.**
**[3]Professor, Department of Pathology, Government Medical College, Bolangir, Odisha, India.**

## ABSTRACT

**Introduction:** Cytodiagnosis of serous effusions relating to distinction between malignant and highly reactive mesothelial cells often possesses diagnostic challenge by routine diagnostic procedures. However, ancillary techniques like immunocytochemistry using panel of antibodies, help in increasing diagnostic accuracy. A combination antibody panel comprising of mesothelial and epithelial cell markers is suggested by various studies to provide distinction between malignant mesotheliomas and adenocarcinomas in serous effusions. Cell block in conjunction with immunohistochemistry can ease the process of more accurate diagnosis. Carletinin, a 29 kd calcium binding protein, normally expressed in neurons is a well recognised immunomarker of mesothelial cells.

**Materials and Methods:** A total number of 504 cases of effusion cytology were studied during the period from August 2015 to August 2017. The fluids were first subjected to routine conventional staining procedures followed by staining with immunostains like carletinin and CEA in diagnostically difficult cases. Cell block preparations were done wherever possible. The results were calculated and tabled.

**Results:** Out of the 504 cases studied, pleural effusion samples outnumbered peritoneal fluid samples. Tuberculosis was the most common cause of non - malignant effusions; adenocarcinoma of lungs and GIT were most commonly encountered entities in pleural and peritoneal fluid samples respectively.

**Conclusion:** For more meaningful comparative studies, the combination of carletinin and CEA in differentiating reactive mesothelial cells and malignant cells were helpful. Utility of immunocytochemistry in cytodiagnosis of malignant effusion was highly significant as compared to the conventional smear methods.

**Key words:** Carcinoembryonic Antigen, Carletinin, Effusion, Serous.

***Correspondence to:**

**Dr. Gayatri Rath,**
D/16, Capital Hospital Campus,
Unit-6, Bhubaneswar, Odisha, India.

**Article History:**

**Received:** 26-01-2018, **Revised:** 28-02-2018, **Accepted:** 30-03-2018

| Access this article online | |
|---|---|
| Website: www.ijmrp.com | Quick Response code |
| DOI: 10.21276/ijmrp.2018.4.2.008 | |

## INTRODUCTION

Cytological examination of pleural and peritoneal fluids is a routine diagnostic procedure in most laboratories and is significant for its diagnostic, therapeutic and prognostic implications. Effusions are of diagnostic challenge for the pathologist, because of the difficulty in distinguishing between adenocarcinomatous cells and reactive mesothelial cells. Sometimes the presence of inflammatory cells and paucity of representative cells in the sample, makes the definitive diagnosis more difficult by light microscopy using routine conventional staining methods. An accurate diagnosis of malignancy in serous effusions is crucial for therapy and thereby modify the prognosis. Hence ancillary techniques, particularly immunocytochemistry is a supplement to the cytomorphological diagnosis. Carletinin, an immunostain expressed normally in neurons of central and peripheral nervous system has assumed a pioneer role, in enabling differentiation between benign reactive mesothelial cells from malignant cells.[1] Carcinoembryonic antigen (CEA), normally detected in glycocalyx of fetal epithelial cells, is considered an epithelial marker with strong staining in adenocarcinomas. In addition, cell block preparations from the fluid sample carries an advantage of studying multiple sections by routine staining. Also, immunohistochemistry, by using commercially available antibody panel on histopathologic sections, help in establishing a definitive diagnosis in problematic cases.

In our study, Carletinin and CEA immunomarkers were used, along with cell block preparations done, wherever possible and also in diagnostically challenging cases.

We undertook this study, to know the diagnostic utility of immunomarkers in serous effusions, particularly of carletinin, that can differentiate between benign and malignant effusions.

## MATERIALS AND METHODS

This is a prospective study conducted in the department of Pathology, SCB medical College from August 2015 to August 2017 in collaboration with other clinical departments. Patients of

variable age and both sexes with absolute indications for fluid cytology were studied. Out of the total number and 894 fluid cytology cases received, 504 cases comprising of pleural, peritoneal and pericardial fluids were included and rest were excluded. About 8-15 ml of fresh fluid was received from each case in a sterile labeled container. Patient's name, age, sex, nature and volume of supplied specimen, nature of effusion (clear, turbid, hemorrhagic), history of recurrent effusion if any, relevant previous history were noted down. Routine and relevant investigation findings, special staining results, were noted down and tabulated. Cell block preparation was undertaken only in selective cases. Microsections were stained with routine Hematoxylin and Eosin stain and immunohistochemistry was done by standard protocol.

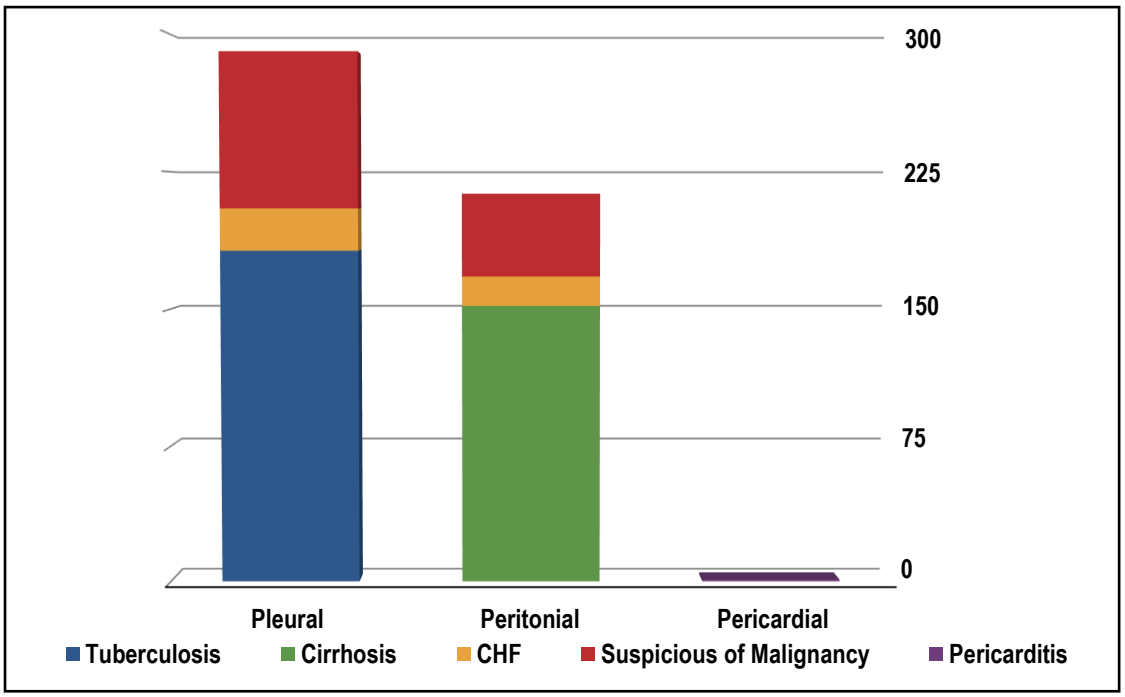


**Table 1: Results of Carletinin and Final Diagnosis**

| Calretinin | Benign | Malignant | Total |
|---|---|---|---|
| **Positive** | 85 | 00 | 85 |
| **Negative** | 06 | 39 | 45 |
| **Total** | 91 | 39 | 130 |

**Table 2: Results of CEA and Final Diagnosis in cases studied**

| Cytology + ICC | Benign | Malignant | Total |
|---|---|---|---|
| **Positive** | 0 | 30 | 30 |
| **Negative** | 91 | 09 | 100 |
| **Total** | 91 | 39 | 130 |

## RESULTS

Out of the total number of 504 cases studied, males (268) outnumbered females (236) and the age range was between 40 to 70 years. Maximum cases were pleural fluids 290(57.6%) cases followed by peritoneal 213(42.3%) cases and one pericardial fluid. Pleural fluids were nonmalignant in 205(70.7%) cases and rest 85(29.3%) cases included either frankly malignant or suspicious of malignancy, which needed confirmation by other special stains. Among peritoneal fluid samples, 168 (78.8%) cases were nonmalignant, while rest 45(21.1%) cases were either malignant effusions or fluids containing suspicious looking cells which needed confirmation by special stains. The only pericardial effusion was nonmalignant. Among pleural effusions, in nonmalignant category, tuberculosis was most common, comprising of 182(88.8%) cases followed by congestive heart failure 23 (11.2%) cases (Fig-1). These 85 pleural and 45 peritoneal fluids were subjected to both carletinin and CEA immunostains. Carletinin was negative in 39 (30%) fluids containing adenocarcinomatous deposits, whereas, the mesothelial cells in the background wherever present took positive staining and were considered as internal control. Six nonmalignant cases were falsely negative for carletinin. CEA stains were positive in 30 (23.1%) malignant cases and negative in 91(76.9%) cases. 9 (7.0%) cases were falsely negative for CEA. However, combining both carletinin and CEA immunostained results, 39 (30%) cases were reported to be confirmed cases of adenocarcinomatous deposits. In malignant category,

adenocarcinoma lungs was the most common 24 (61.5%) cases, followed by breast 7 (17.9%) cases, adenocarcinoma of GIT comprised of 5 (12.8 %) cases and malignant ovarian tumour in 3 (2.6%) cases. Cell block preparations were done in only 6 cases suspicious of malignancy. All these cases were found out to be malignant.

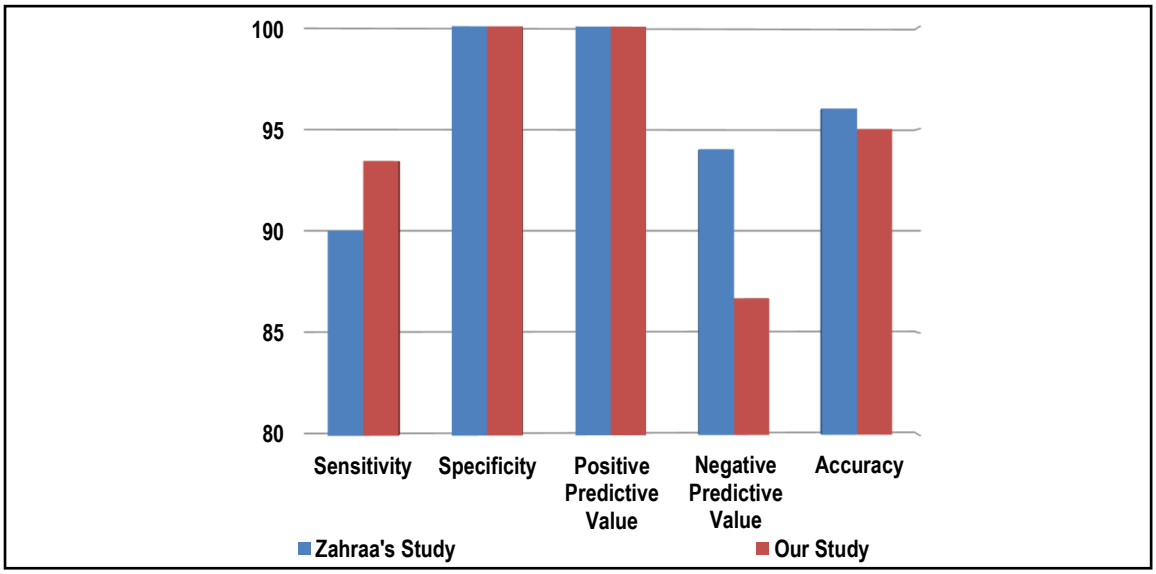


**Fig 2: Correlation of Carletinin ICC Staining Results Between our Study and Zahraa's Study**

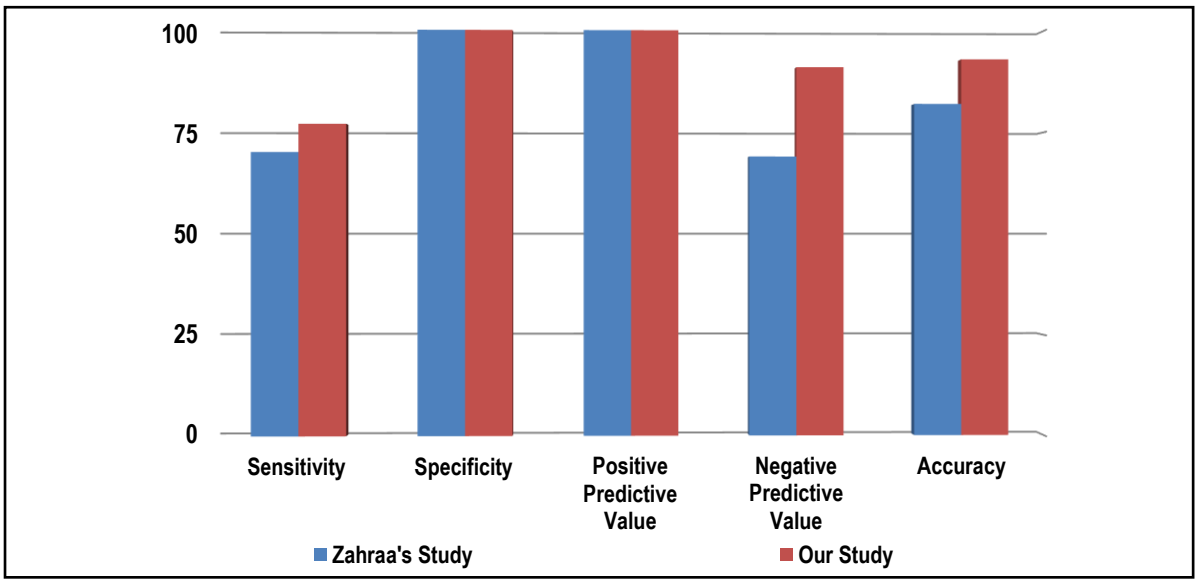


**Fig 3: Correlation of CEA ICC Staining Results Between Our Study And Zahraa's Study**

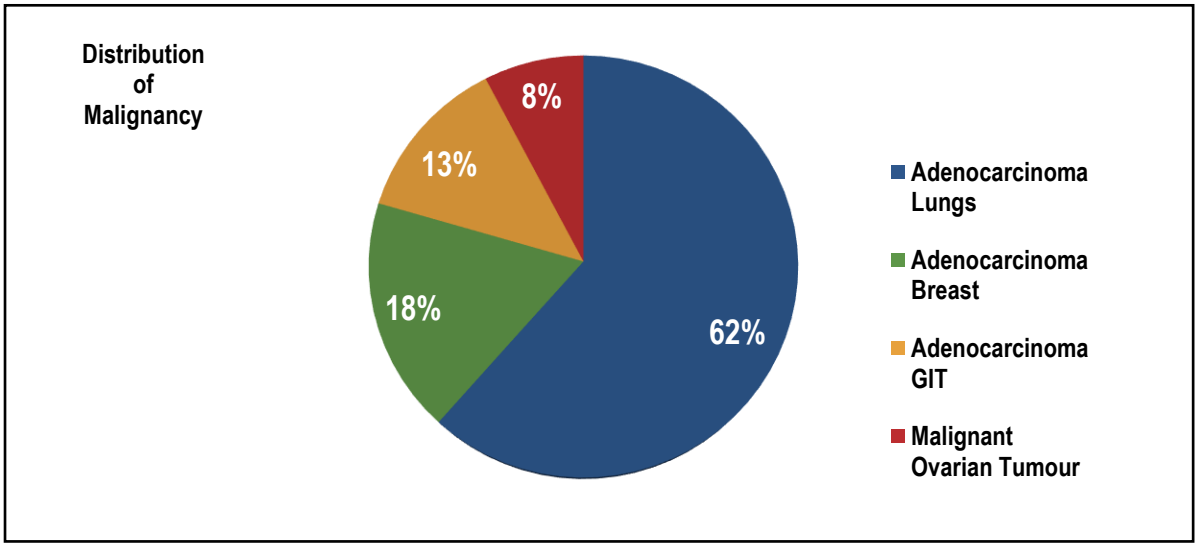


**Fig 4: Distribution of Malignancy in Cytology Smears**

**Table 3: Comparison of ICC (Smear) and IHC (Cell Block) (Total 39 Cases)**

| Malignant Cells In Effusion | Benign | Atypical Cells | Malignant |
|---|---|---|---|
| ICC | 0 | 6 | 33 |
| IHC | 0 | 0 | 39 |

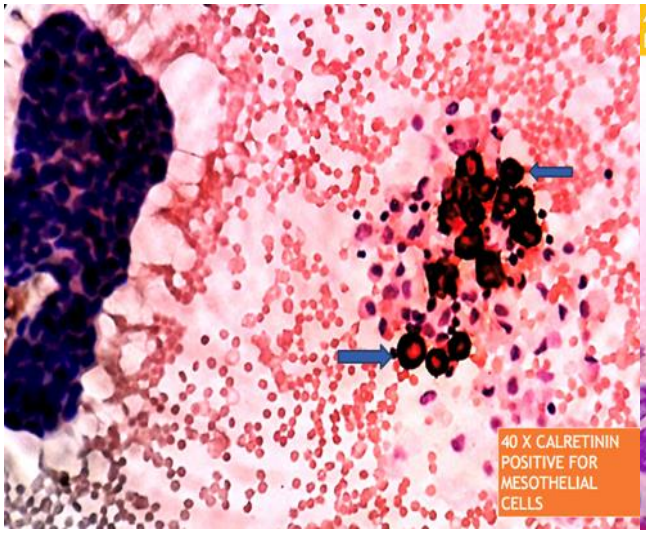


**Fig 5: ICC Staining for Carletinin.**

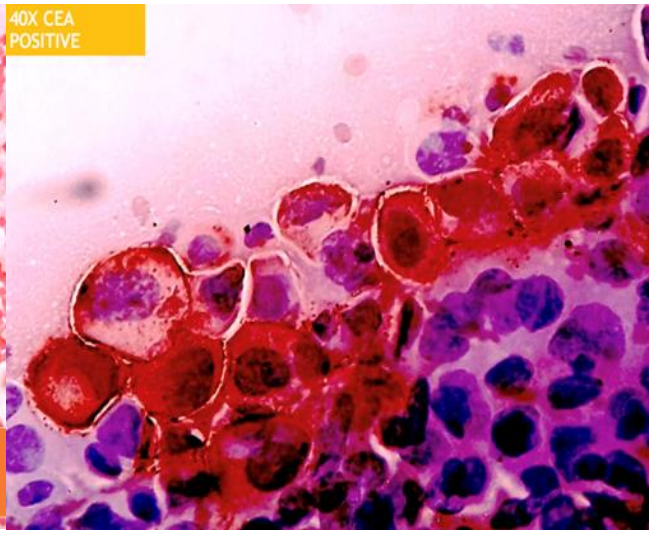


**Fig 6: ICC Staining for CEA**

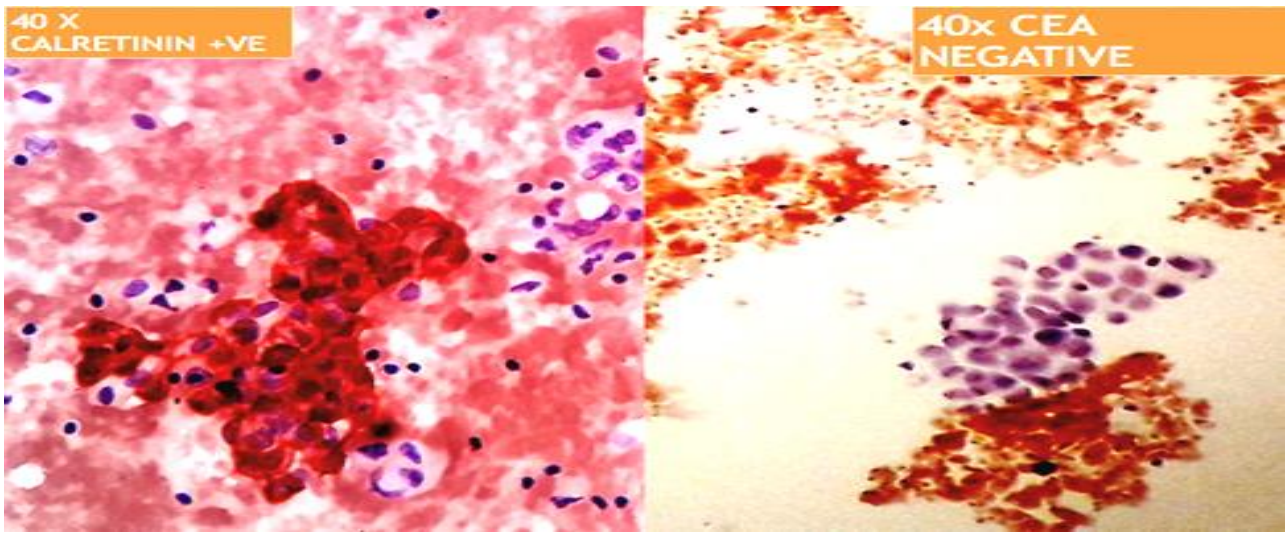


**Fig 7: Comparative results between CEA and Carletinin**

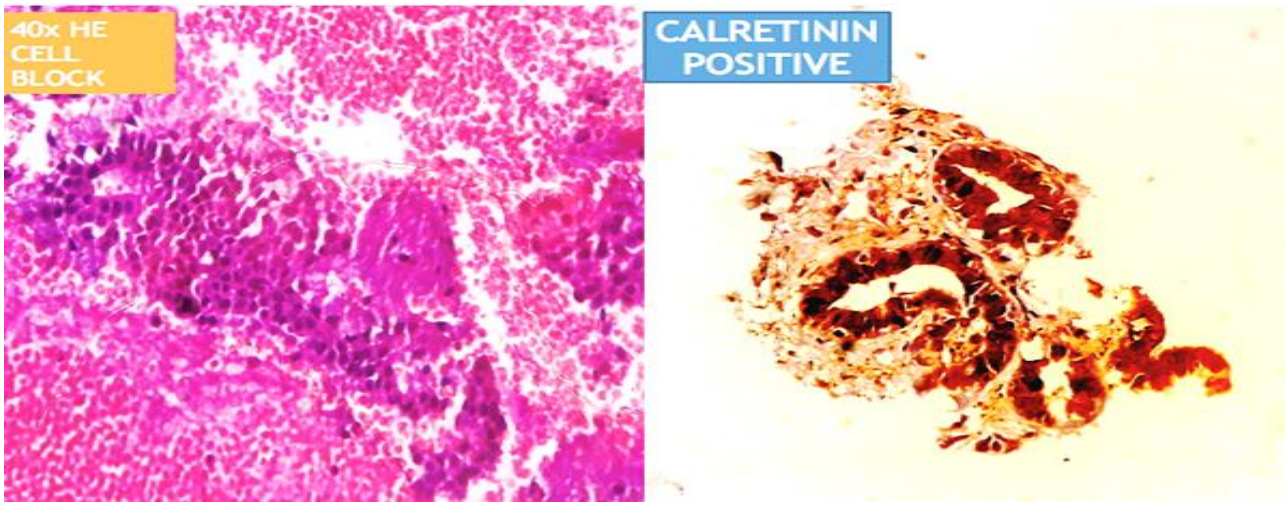


**Fig 8: Cell Block Preparation**

## DISCUSSION

Serous effusions are commonly encountered in certain inflammatory conditions most common being tuberculosis, noninflammatory conditions like cirrhosis and congestive cardiac failure, various organ system malignancies and malignant mesotheliomas. Reliable identification of the primary tumour origin from malignant effusions is important for tumour staging, treatment and prognosis. Distinction between malignant mesothelioma and adenocarcinoma cells cytomorphologically in body cavity effusions is one of the most challenging problem in effusion cytology.[2] Although biopsy is the gold standard for a confirmatory diagnosis, this is an invasive procedure, may complicate the disease process by tumour cell seedling or may be infeasible because of poor condition of the patient.[3] Tumour biomarkers, being a noninvasive technique help in arriving at a conclusive diagnosis. So to improve the diagnostic accuracy, panel of tumour markers are being used today. Selection of antibody for early and accurate diagnosis of malignant effusions is important. The present study included 504 cases of effusions, which were referred from different clinical departments during the period from August 2015 to August 2017. A majority of patients were in the age group of 40 to 70 years, similar to that of Anurag Agrawal (median age 58.8 years; range 32-85 years).[4] Male to female ratio was 1.7:1. Incidence of malignancy in males in the present study was higher probably due to several factors including genetic differences, environmental causes and occupational exposure including smoking, diet, sunlight exposure etc. In our study, majority of cases were pleural effusion samples, followed by peritoneal fluids, which was same as that of Luse and Reagan.[5] We detected malignancy in 39 (34.21%) cases out of the 114 (22.61%) hemorrhagic samples received. Similar findings were noted by Melamed who reported malignancy in one third of hemorrhagic fluids.[6] Out of the total number of 290 (57.5%) pleural fluids received, 205 (40.7%) cases were frankly nonmalignant comprising mostly of tubercular 182(88.8%) cases, followed by congestive heart failure 23 (11.2%) cases. Tuberculosis is always a leading cause of pleural effusions in developing countries like India. Among the total number of 213 (42.3%) peritoneal fluids received, 168 (78.9%) cases were frankly nonmalignant, comprising of cirrhosis 152 (90.5%) cases and congestive heart failure 16 (9.5%) cases. All the 85 pleural fluids and 45 peritoneal fluids, either confirmed, or suspicious of malignancy were subjected to both carletinin and CEA immunostains. Carletinin, a 29kDa calcium- binding protein is expressed in central and peripheral nervous tissue, also in mesothelium, endometrium and adrenal cortical cells. It is strongly reactive in benign and malignant mesothelial cells with a strong cytoplasmic and nuclear staining pattern (Fig-5). It has proved to be a useful immunocytochemistry marker for distinguishing malignant or reactive mesothelial cells from adenocarcinoma cells. Control for carletinin was normal appendix and that of CEA was adenocarcinoma colon. Carletinin was negative in all 39 adenocarcinomatous deposits. However 6 nonmalignant cases were falsely negative showing sensitivity of carletinin for detecting mesothelial cells to be 93%, with 100% specificity, a positive predictive value of 100%, a negative predictive value of 87% and 95% accuracy (Table-1, Fig-2). The combined predictive parameters of carletinin of others were at par with the present study. CEA shows diffuse cytoplasmic staining with membrane enhancement in adenocarcinoma.(Fig-6) Immunostaining for CEA was positive in adenocarcinoma cells in 30 out of 39 cases with 9 false negative results. The sensitivity of CEA for adenocarcinoma cells b; was 76%, with 100% specificity, a positive predictive value of 100%, a negative predictive value of 91% and an accuracy rate of 93% (Table-2, Fig-3). Thus combining the results of both the immunostains, 39 cases were concluded to be malignant (Fig-7). In malignant category, adenocarcinoma lungs comprised of 24 (61.5%) cases, followed by breast 7 (17.9%) cases, GIT 5 (12.8%) cases and malignant ovarian tumour cell infiltration in 3 (2.6%) cases (Fig-4). Lungs carcinoma accounted for about 61.5% of malignant effusions similar to the study of Hauseer, which were 65% of malignant pleural effusions.[7] Thus, the results of the current work, as well as of others have shown carletinin as a reliable and specific marker for mesothelial cells. Similar results were found with Zahraa Mohammed Yahya.[8] When both routine cytological examinations of effusion fluids were considered and immunocytochemical results correlated, an increase in sensitivity, specificity and accuracy of effusion diagnosis was observed. When a positive staining for CEA and a negative staining for carletinin were considered as an indication of malignancy, the sensitivity of cytological and immunocytochemical results was shown to be increased from 76% to 84%. Due consideration was given to age, sex, site of effusion, clinical and radiological findings, to arrive at a final diagnosis and to identify the primary malignant lesion. Cell blocks provide diagnostic information complementary to that obtained from cell smear examinations.[9] (Fig-8). When we added cell block methods, it helped in arriving at a confirmatory diagnosis. All the 6 cases suspicious of malignancy came out to be malignant (Table-3). It further increased the sensitivity from 84% to 90%. Ceelen got 89% positive diagnoses with cell block technique.[10] Taft et al also compared cell block technique with smear examination and concluded that cell block technique yielded better results than smears.[11] So combining the cytological smears with immunostaining and cell block methods the sensitivity and accuracy of finding the primary site of origin of the tumour, differentiation between benign and malignant tumours was made easy.

## CONCLUSION

Use of immunomarkers in addition to conventional routine staining procedures, has contributed to an increase in diagnostic accuracy. It necessitates the obvious need in worldwide cytological practice to make a final diagnosis by employing panel of antibodies in conjunction to morphological diagnosis using routine staining procedures. Carletinin is both a sensitive and specific marker of reactive and neoplastic mesothelial cells.

## REFERENCES

1. Rogers, J. H. Carletinin: a gene for a novel calcium binding protein expressed principally in neurons. J Cell Biol 105, 1343-1353 (1987).

2. Jo V. Y., Cibas E.S. & Pinkus G. S. Claudin-4 Immunohistochemistry is highly effective in distinguishing adenocarcinoma from malignant mesothelioma in effusion cytology. Cancer Cytopathol 122, 299-306 (2014).

3. Kastelik JA. Management of malignant pleural effusion. Lung 191, 165-175 (2013).

4. Agrawal A, Tandon R, Singh L et al. Clinics-pathological profile and course of malignant pleural effusion in a tertiary care teaching hospital in western U. P. with special reference to lung cancer. Lung India. 2015; 32(4):326-330.
5. Luse SA, Reagan JW. A histocytologic study of effusions: Effusions associated with malignant tumours. Cancer. 1954; 7: 1167-81.
6. Melamed MR. The cytological presentation of malignant lymphomas and related diseases in effusions. Cancer. 1963; 16: 413-31.
7. Hauseer FH, Yarbro JW: Diagnosis and treatment of malignant pleural effusion. Semin Oncol 12:54, 1985.
8. Yahya ZM, Ali HH, Hussein HG. Evaluation of the sensitivity and specificity of Immunohistochemical markers in the Differential Diagnosis of Effusion Cytology. Oman Med J. 2013; 28(6):410-16.
9. Thapar M, Mishra RK, Sharma A, Goya's V. Critical analysis of cell block versus smear examination in effusions. J Cytol. 2009; 26:60-4.
10. Ceelen GH: The cytologic diagnosis of ascetic fluid. Acta Cytol. 1964; 8:175-85.
11. Taft PD,Kenisha MJ, Naylor B. Cells of Squamous cell carcinoma in pleural, peritoneal and pericardial fluids: Origin and morphology. Acta Cytol. 1960; 33:245-53.

**Source of Support:** Nil.

**Conflict of Interest:** None Declared.



**Cite this article as:** Shilpa Dash, Gayatri Rath, Lity Mohanty. Diagnostic Utility of Immunocytochemistry in Serous Effusions. Int J Med Res Prof. 2018 Mar; 4(2):34-39.
DOI:10.21276/ijmrp.2018.4.2.008