

Cytologic analysis of body fluids with an emphasis on malignant effusions

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Abstract

Introduction: Fluid cytology, a relatively non invasive technique, throws light on both malignant and non malignant causes of effusions. It especially contributes in cancer research and staging of various tumors.

Materials and Methods: This was a retrospective study done over a period of two years from January 2014 to December 2015 in the department of Pathology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad. The body fluids included in the study were pleural, peritoneal, pericardial and synovial fluids. All other fluids were excluded. The relevant clinical data was noted. Gross, cell count and cytomorphological examinations of fluid were done.

Results: Out of 302 cases, pleural fluids, 148 (49.0%) cases, were most common, followed by peritoneal fluid, 125 (41.39%) cases and least common was pericardial fluid, 8 (2.64%) cases. The maximum numbers of cases was in 5th decade. The age range was 3 years to 84 years. Female preponderance was observed with M: F ratio of 1: 1.17. Lymphocyte rich exudates were most common in pleural effusion. In peritoneal and pericardial effusions, transudates were more and suppurative inflammation was predominant in synovial fluid analysis. Over all, malignant effusions constituted 18.54% of effusions. Metastatic adenocarcinoma was the commonest malignancy detected in all body cavity effusions.

Conclusions: Fluid cytology is relatively painless, simple, cost effective, rapid technique that yields quick and reliable results. Some cases may present major interpretative challenges to the pathologist. Ancillary studies such as cell count, biochemical, microbiologic evaluation, cell block and immunohistochemistry (IHC) study help in accurate identification of diagnosis.

Keywords: Body fluids, Malignant effusions, Transudates.

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Introduction

Effusion cytology is study of cells within the fluids of serous cavities, mainly the pleural, peritoneal and pericardial fluids. It is a useful means of studying the different pathologic processes and thereby elucidating the cause of effusion.^{1,2} The diagnostic yield of effusion fluid is higher than needle biopsy since the cell population present in the sediment is representative of a much larger surface area.³⁻⁵ On differentiating effusion into transudates and exudates one can identify the underlying pathological process there by guiding the further investigations. The most important and also challenging task of effusion cytology is detection of malignant cells which helps in staging of malignancy and also in monitoring of response to therapy.⁶ This study was carried out to know the trends of various types of effusions diagnosed in a tertiary care centre with an emphasis on malignant effusion.

Materials and Methods

The present study was carried out in the department of Pathology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad from January 2014 to December 2015. All the cases of neoplastic and non neoplastic diseases with effusion of pleural, peritoneal, pericardial and synovial cavity received in the department during that period were studied. Other fluids were excluded. All the relevant clinical, radiological, biochemical data were obtained. The gross findings were noted, cell count was done in improved Neubaur chamber, then centrifuged at 2000 rpm for 5 min. For hemorrhagic fluids, glacial acetic acid was used as a hemolysing agent and then processed routinely. Cytospin was also used for some samples. Smears were made from the sediment. Both wet fixed and air dried smears were prepared and stained with hematoxylin and eosin, and May Grunwald Giemsa stains respectively. In cases where the cell block was requested, the sediment was fixed in formalin

and processed like a routine histopathology specimen. Immunohistochemistry (IHC) was done, wherever required, using makers like cytokeratin 7, cytokeratin 20, CA 125, TTF1, etc.

Results

A total of 302 cases of serous effusions were examined cytologically which included pleural, peritoneal, pericardial and synovial fluids.

Table 1: Age and gender wise distribution of cases

Age	Pleural fluid		Peritoneal Fluid		Synovial fluid		Pericardial fluid		Total		Grand total
	M	F	M	F	M	F	M	F	M	F	
0-10	1	0	0	0	0	0	0	0	1	0	1
11-20	6	7	1	2	0	1	0	0	7	10	17
21-30	21	10	0	9	1	4	0	1	22	24	46
31-40	6	6	4	6	1	2	0	1	11	15	26
41-50	19	7	7	32	3	1	3	1	32	41	73
51-60	9	8	13	26	2	1	1	1	25	36	61
61-70	21	6	3	12	1	3	0	0	25	21	46
> 70	9	12	6	4	1	0	0	0	16	16	32
Total	92	56	34	91	9	12	4	4	139	163	302
Grand total	148		125		21		8		302		

The age ranged from 3 years to 84 years. Female preponderance was observed with M: F ratio of 1:1.17. The most common fluid was pleural, 148 (49.0%) cases, followed by peritoneal fluid, 125 (41.39%) cases and least common was pericardial fluid, 8 (2.64%) cases. The maximum number of cases were seen in the 5th decade.

Table 2: Distribution of effusions on cytological examination

Type of fluid	Transudate	Exudate					Total
		Neutrophil rich effusion	Mixed inflammatory infiltrate effusion	Lymphocyte rich effusion	Malignancy	Susp of Malignancy	
Pleural	13	7	19	85	21	3	148
Peritoneal	50	7	25	9	33	1	125
Synovial	0	14	7	0	0	0	21
Pericardial	4	0	0	2	2	0	8

Out of 148 cases of pleural effusion, lymphocyte-rich exudates were most common, 58.62% followed by malignant effusion 14.18 %. Transudates constituted 8.75%.

Out of 125 cases of peritoneal fluids, the maximum numbers of cases were transudates, 40%, followed by malignant effusions, 26.4%.

In synovial fluids analysis (n= 21), the maximum cases were of acute suppurative inflammation 66.66 %, followed by acute on chronic inflammation, 33.33%.

Out of 8 cases of pericardial effusion, 50% were transudates, and 2 cases each of malignant effusion and lymphocyte rich effusions were seen.

Table 3: Malignant effusion

Type of fluid	Ca Ovary	Ca Lung	Ca Breast	Ca Stomach	SCC Cervix	Ca GB	MMMT Endome-trium	Unknown primary	Suspi Malignancy	Total
Pleural	3	11	4	0	1	0	0	2	3	24
Peritoneal	27	0	0	2	1	1	1	1	1	34
Pericardial	0	1	1	0	0	0	0	0	0	02
Total	30	12	5	2	2	1	1	3	4	60

Ca - Adenocarcinoma

SCC - Squamous cell carcinoma

GB - Gall bladder

Suspi - Suspicious

Out of 302 cases of effusions, malignant cells were detected definitely in 56 (18.54%) cases. Maximum numbers of malignant effusions were peritoneal, 60.71% followed by pleural 42.85%. Metastatic adenocarcinoma was the commonest malignancy detected in all effusions. The commonest cause of malignant peritoneal and pleural effusions was metastatic adenocarcinoma ovary (79.41%) and adenocarcinoma lung (45.83%) respectively.

Four cases were suspicious of malignancy. These fluids were slightly hemorrhagic and showed either singly scattered bizarre cells or occasional clusters of atypical cells with high nuclear cytoplasmic ratio. Cell block also revealed the same. Unfortunately there was no follow up.

Table 4: Comparison of our study results with similar studies.^{5,7,11}

Studies	Pleural effusions			Peritoneal effusions			Pericardial effusions			Synovial effusion			Total
	Non malignant (%)	Malignant (%)	Total	Non malignant (%)	Malignant (%)	Total	Non malignant (%)	Malignant (%)	Total	Non suppurative (%)	Suppurative (%)	Total	
Shulbha et al	93.6	6.4	94	97.7	2.3	174	Nil			25	75	8	385
Chakrabarti et al	91.7	8.3	400	90.5	9.5	485	82.4	17.6	17	Nil			902
Kol P.C et al	77.6	22.4	76	77.7	22.3	103	100	0	01	Nil			180
Present study	83.8	16.2	148	72.8	27.2	125	75	25	08	33.3	66.6	21	302

Discussion

The cytological examination of effusion fluids in body cavities is a simple procedure and yields vital information of the cell population involving the cavities thereby suggesting the etiology. In cases of malignancies it helps in staging of the malignancy.¹⁻³

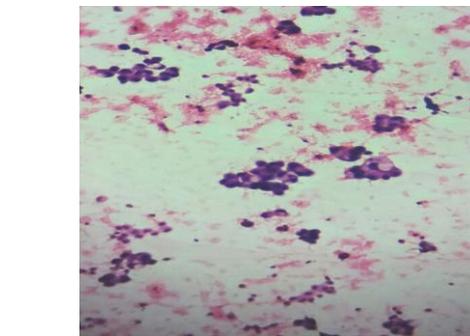
In our study the most common effusion was pleural, 49.0%, followed by peritoneal effusion 41.39%, which compares well with the study by Kumavat et al⁶ but is in contrast with the study by Chakrabarti et al⁵ who found peritoneal effusions more than pleural effusions.

In pleural fluid analysis, maximum numbers of cases were seen in 4th decade followed by 7th decade which is in correlation with Chakrabarti

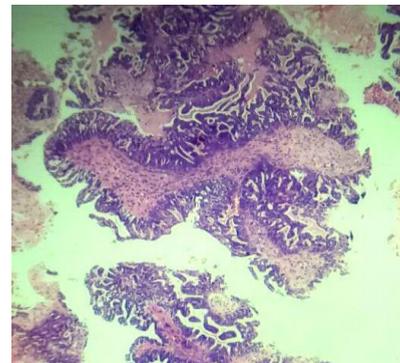
et al study, in which the majority of pleural effusion cases were in the 4th decade. Male preponderance (2.1:1) seen in our study was also observed by the above authors. The effusions with total protein more than 3gm were taken as exudates. Of all the pleural effusions, lymphocyte rich exudates were the most common effusions, 85 (58.62%) cases followed by malignant effusions 24, (16.21%) cases. Kumavat et al⁶ reported similar observation but Chakrabarti et al⁵ and Shulbha et al⁷ observed transudates as most common effusions. These lymphocyte rich exudates were clinically suspected as tuberculosis. On cytomorphological examination these effusions revealed cells more than 500 cells/ cu mm with more than 50% mature lymphocytes and less than 1%

mesothelial cells. It correlates well studies by Kushawaha⁸ et al and Tetikkurt et al.⁹ Though we did AFB staining in all cases, none was positive. The second most common pleural effusions were malignant effusions, primary adenocarcinoma lung being the most common etiology. Next in frequency was carcinoma breast followed by carcinoma ovary which is in agreement with Chakrabarti et al⁵ and other authors.^{8, 9, 10} We had three cases that were suspicious of malignancy and 2 cases of unknown primary malignancy. Further workup in these cases was not available. Most of the transudates were due to congestive heart failure (CHF), cirrhosis of liver, renal failure or hypoproteinemia. All neutrophil rich effusions (empyema) were due to either pneumonia, post myocardial infarction and the clinical diagnosis was already obvious in such cases. In cases of empyema, the fluid was thick; white to yellowish and contained numerous viable as well as degenerate neutrophils. Similar findings were seen in the study by Kumavat PV et al.⁶

In peritoneal fluid analysis, maximum cases were observed in 6th and 7th decades but Chakrabarti et al⁵ found maximum cases in 4th decade. Female preponderance was noted (1:2.6) in our study, which is in agreement with above study. Out of the 125 cases of peritoneal fluid, transudates were the predominant effusions, (40%), which was similar to many studies.^{5-7, 11} Cirrhosis of liver was the commonest etiology. In these cases there were variable infiltrates of lymphocytes, histiocytes and protein was less than 3 gm. Next common causes of effusions were malignancies, 33 (26.4%) cases, with 90% being due to primary adenocarcinoma of ovary (Fig 1). Kumavat et al,⁶ Chakrabarti et al⁵ and others reported similar findings.^{5, 6, 10, 11} This may be due to female preponderance and adenocarcinoma ovary is most common malignancy in females. Rest of the malignant peritoneal effusions was due to, squamous cell carcinoma cervix, adenocarcinoma gall bladder (Fig. 2), malignant mixed Mullerian tumor of endometrium and unknown primary, each one case. Jha et al¹² found adenocarcinoma stomach as most common primary carcinoma affecting peritoneum but we had only two cases of carcinoma stomach affecting the peritoneal fluid.



(A)



(B)

Fig. 1: Metastatic ovarian papillary serous cystadenocarcinoma. (A): Malignant cells in cohesive clusters and papillae in ascitic fluid (H & E, 100X); (B): Corresponding histopathology of ovarian carcinoma in the same case shown in A (H & E 100X)

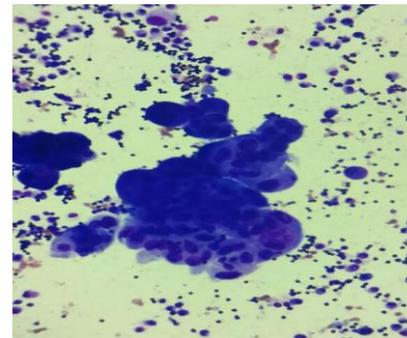


Fig. 2: Smear showing three-dimensional clusters of malignant cells in clinically diagnosed case of carcinoma gall bladder (MGG 100X)

In synovial fluid analysis, maximum cases were seen in 3rd decade and female preponderance was observed. Majority of effusions were suppurative in nature having characteristic turbid, thick, yellowish fluid and were clinically correlated with acute arthritis. Remaining cases were also exudative in nature but revealed mixed inflammatory infiltrate.

Shulbha et al⁷ also found suppurative effusions more than non suppurative effusions.

In the present study pericardial fluids were least in number which is comparable to the study by Kol et al.¹¹ Out of 8 cases three were in 5th decade and had equal gender distribution. Most of pericardial effusions, 4 (50%) cases were transudative in nature and they were clinically suspected cases of pericarditis, post myocardial infarction which is similar to Chakrabarti et al study. In remaining half cases, two were of lymphocyte rich effusion and other two were of malignant effusion, one case each of primary breast and lung adenocarcinoma. Chakrabarti et al⁵ and Robert et al¹³ also observed that the most common cause of malignant pericardial effusion was adenocarcinoma breast.

In the present study, 44 out of 60 cases with malignant effusion already had a known primary malignancy. Remaining cases, presented with effusion only, where cytology picked up malignancy. Of these, cell block, IHC study and correlation with radiology identified the primary malignancy in eleven cases, and three (5%) remained as unknown primary. Fig. 3 demonstrates immunoreactivity for CA 125 in Cell block of ascitic fluid indicating the possibility of carcinoma ovary. Cases of suspicious of malignancy were lost to follow up. Shulbha et al and Luse et al in their study found 40%, and 15% respectively, as overall rate of unknown primary. In addition to cytomorphology, immunocytochemistry, clinical correlation and follow up help in detecting the primary malignancy.^{15, 16}

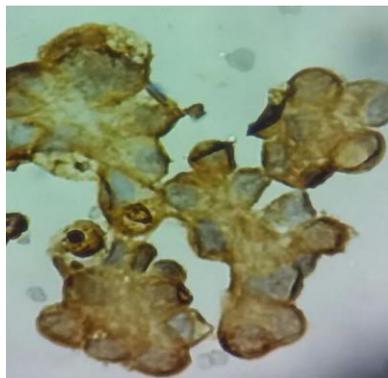


Fig. 3: Immunoreactivity of tumor cells for CA 125 on cell block preparation (400X)

In some inflammatory effusions, we encountered difficulties in identifying reactive mesothelial cells and distinguishing them from malignant cells. However, the morphologic changes in these cells were not sufficient enough to be concluded as malignant. We applied a "two cell population" approach in identifying

metastatic tumor cells in addition to cytomorphology and ancillary studies such as immunocytochemistry wherever necessary. Reactive mesothelial cells are identified by the presence of two zones in cytoplasm, cohesive clusters with scalloped (knobby) contours, cell-in-cell configuration and mesothelial windows.^{2,17,18}

Conclusions

We conclude in our study that cytology is a valuable tool in evaluation of serous cavity fluids. It is relatively painless, simple, cost effective, rapid technique that yields quick and reliable results. Some cases may present major interpretative challenges to the pathologist. Ancillary studies such as cell count, biochemical, microbiologic evaluation, cell block and IHC study help in accurate identification of diagnosis. It is especially helpful in evaluating and staging malignancies thereby guiding the clinician in further management.

In our study, pleural fluid was the commonest type of serous fluid in which the majority of cases were lymphocyte-rich effusions favouring tuberculosis. Adenocarcinoma was the most common malignancy involving serous cavities. In transudates, the diagnosis helped the clinician in evaluating the causes and in follow up of the cases.

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