



## To Study Cytological Patterns, both Benign and Malignant in Pleural and Peritoneal Fluids

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### Abstract

**Aim:** To study cytological patterns, both benign and malignant in pleural and peritoneal fluids and to correlate cellular findings with clinical diagnosis

**Materials and Methods:** The study was conducted in the Department of Pathology, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu (J&K), over a period of one year (Nov.1, 2014 to Oct. 31, 2015). All clinical information regarding age, sex, symptoms and accompany signs, various pathological, biochemical and radiological findings was recorded in a pre structured proforma. Fluids were examined grossly followed by smear preparation. Staining of the slides by Papanicolaou stain and Hematoxylin and Eosin stain (H&E) was done and whenever required special staining like Periodic acid Schiff (PAS) and Alcian blue-PAS was performed.

**Results:** A total of 140 serous fluids were studied. The youngest patient was 7 years old and the oldest was 80 years old. Male to female ratio was 1.3:1. Of the serous fluids studied, 73 (52.15%) were pleural fluids, 67 (47.85%) were peritoneal fluids. 20% of the serous fluids studied were cytologically malignant and 80% were cytologically benign. 62.14% of the serous fluids were exudative and 37.86% were transudative in nature. Of all the cytologically malignant cases, 85.71% were exudative and 14.29% were transudative in nature. Among the cytologically non-malignant cases, 56.25% were exudative and 43.75% were transudative in nature. The non-malignant effusions were chiefly caused by Congestive cardiac failure (18.75%), Cirrhosis of liver (13.40%) and tuberculosis (12.50%). Most common causes of transudative non-malignant effusion were CCF, Cirrhosis of liver, nephritic syndrome and pneumothorax. Tuberculosis, renal failure, pneumonia, peritonitis, COPD, acute cholecystites, appendicitis, hepatic abscess, pancreatitis, empyema and pelvic abscess constituted the common causes of exudative non-malignant effusions in decreasing order of frequency. Among the 28 malignant cases 82.14% were adenocarcinoma, 7.14% as squamous cell carcinoma and lymphoma each and 3.57% as HCC. Lung was the most common primary site for malignant pleural effusion in case of males. In females, lung and breast both shared being the most frequent primary site. In case of peritoneal effusions, GIT formed the most frequent primary site in case of males and ovary in females.

**Conclusions:** The cytological study of body effusions is a complete diagnostic modality which aims at pointing out the etiology of effusions as well as in certain cases a means of prognostication of the disease process. Further studies need to be carried out including large number of cases along with application of newer technologies in the evaluation of effusions regarding etiology and in case of malignant effusions to determine the primary site. In the recent years with the availability of several antibodies, use of immunohistochemistry (IHC) and even molecular genetics on these fluid specimens, accurate diagnosis can be achieved and the typing of malignant cells has become more reliable.

## Introduction

Body fluids like peritoneal fluids and pleural fluids are normally present within the serous body cavities with their constituents in particular proportions and in minimal quantities. These fluids, however, undergo abnormal qualitative and quantitative changes in various pathological conditions and often represent the earliest evidence of an underlying disease. A tuberculous pleural effusion is frequently the only clinical manifestation of that disease.

Aspiration of these fluids is a simple and relatively non-invasive technique to achieve a diagnosis. Thus, the cytological study of body effusions is a complete diagnostic modality which aims at pointing out the etiology of effusions as well as in certain cases a means of prognostication of the disease process. Moreover, the diagnostic performance of the cytological study of fluid may be attributable to the fact that the cell population present in the sediment is representative of a much larger surface area than that obtained by needle biopsy<sup>1</sup>. Cytological analysis of the body fluids reveals information about inflammatory conditions of the serous membranes, parasitic infestations, infection with bacteria, fungi and viruses<sup>2</sup>.

Benign effusions are twice as common as malignant effusions and have diverse causes and manifestations which often make them a diagnostic challenge. Differentiating effusions as transudate or exudate is the first, and often helpful, step in directing investigations for diagnosis and management. Congestive heart failure (CHF), cirrhosis of liver, nephrotic syndrome are the known causes of transudative effusion while as infections like tuberculosis (TB), malignancy, collagen diseases viz lupus or rheumatoid arthritis (RA) account for the causes of exudative effusion.

The cytological diagnosis of malignant tumor cells in serous effusions is an important diagnostic test, for it may be the first positive evidence of a cancer. If a definitive diagnosis can be made, it may save exploratory operations<sup>3</sup>. Malignancy

accounts for around 15% of the effusions. The usual cases of malignant peritoneal effusions are the malignancies of gastrointestinal tract (GIT) like stomach carcinoma, colon carcinoma, primary Hepatocellular carcinoma (HCC) and metastatic liver carcinoma. Lung cancer and breast cancer account for about 50% - 60% of malignant pleural effusions<sup>4</sup>. Lymphomas and pleural mesothelioma are the other causes of pleural effusion. In children, however, the most common cause of a malignant pleural or peritoneal effusion is non-Hodgkins lymphoma<sup>5</sup>.

In the recent years with the availability of several antibodies, use of immunohistochemistry (IHC) and even molecular genetics on these fluid specimens, accurate diagnosis can be achieved and the typing of malignant cells has become more reliable.

The present study is based on analysis of cytologic findings in pleural and peritoneal fluids examined in the Department of Pathology ASCOMS and Hospital over a period of one year (Nov. 1, 2014 to Oct. 31, 2015 inclusive). It demonstrated usefulness of cytology in differential diagnosis of pleural effusion and ascitis, particularly those caused by cancers. The cellular findings were correlated with the clinical diagnosis.

## Aims and Objectives

- To study cytological patterns, both benign and malignant in pleural and peritoneal fluids.
- To correlate cellular findings with clinical diagnosis.

## Materials and Methods

The present study was undertaken in the Department of Pathology, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu (J&K), over a period of one year (Nov.1, 2014 to Oct. 31, 2015). All clinical information regarding age, sex, symptoms and accompany signs, various pathological,

biochemical and radiological findings was recorded in a pre structured proforma.

Fluids were examined grossly for volume, colour and consistency. This was followed by smear preparation from the fluid specimen. Staining of the slides by Papanicolaou stain and Hematoxylin and Eosin stain (H&E) was done by first dropping the slides into 95% ethyl alcohol for a minimum of 15 minutes prior to staining as they both require wet fixation. For May-Gntnwald Giemsa stain, air dried smear was used followed by fixation in 95% methyl alcohol. Whenever required special staining like Periodic acid Schiff (PAS) and Alcian blue-PAS was performed.

An effort was made in this study to process the fluid specimens as expeditiously as possible. In

case of delay in processing; these specimens were stored in the refrigerator at 4°C.

### Results

Out of 140 serous cavity fluid sample, 73(52.15%) were pleural fluids and 67 (47.85%) peritoneal fluids. The age range in our case was 7 to 80 years. More than two-thirds of all patients were above 40 years of age. 79(56.50%) patients were males and 61(43.50%) were females, with male to female ratio of 1.3:1

Out of 73 Patients presenting with pleural effusions, male preponderance (63%) was noted. In peritoneal fluids the sex distribution was almost equal.

**Table – 1** Showing age and sex distribution of patients with pleural and peritoneal effusions

Age in years	Pleural		Pleural	
	Male	Female	Male	Female
0 – 10	1	1	1	1
11 – 20	4	3	0	1
21 – 30	3	2	2	2
31 – 40	6	3	5	3
41 – 50	8	4	8	6
51 – 60	9	5	6	6
61 - 70	7	5	6	7
71 – 80	8	4	5	8
<b>Total</b>	<b>46</b>	<b>27</b>	<b>33</b>	<b>34</b>

**Table –2** Various types of serous fluid and their distribution into malignant and non-malignant are

S. No	Type of Fluid	Total	Non Malignant	Malignant
1	Pleural Fluid	73	57(78.08%)	16(21.92%)
2	Peritoneal Fluid	67	55(82.08%)	12(17.92%)
	<b>Total</b>	<b>140</b>	<b>112(80%)</b>	<b>28(20%)</b>

From above table, it is observed that out of 140 serous effusions maximum number of cases were non-malignant with malignant cells present in 21.92% and 17.92% of pleural and peritoneal fluids respectively. Majority of patients with malignant effusion were above 40 years of age, with 75% in pleural effusions and 66.7% in peritoneal effusion with equal sex distribution. Two third of patients of pleural effusions and three-fourth of patients of peritoneal effusion were above 40 years of age with male predominance in pleural effusion

Those cases where specific gravity was less than 1015 and protein content less than 3 gm/100 ml were included in transudates and those with specific gravity above 1015 and total protein contents more than 3 gm/100 ml were taken as exudates. Out of 140 serous fluids studied, 87(62.14%) were exudate in nature and 53 (37.86%) were transudative in nature.

Out of 140 Fluids studied 28(20%) were cytologically malignant and 112 (80%) were cytologically benign. Among cytologically malignant cases, 16(57.15%) were pleural effusions and 12 (42.85%) were peritoneal

effusions. Out of 28 cytologically proved Malignant Serous Fluids, 24 were exudative in nature and only 04 were transudative. Out of 112 (80%) cytologically benign effusions, 57(50.90%) were pleural and 55(49.10%) were peritoneal. Out of 112 non-Malignant Serous Fluids, 63 were exudative in nature and 49 were transudative.

Out of total of 140 Patients, edema was found to be the most common clinical features seen in 76 (54.28%) cases followed by weakness in 54(38.6%), dyspnea in 39(27.85%), pallor in

30(21.42%), mass in 26 (18.57%), haemoptysis in 24 (17.14%) and lymphadenopathy in 14(10%) cases. Some patients also presented with pain abdomen, oliguria and vomiting.

Among 112 non-Malignant Effusion, congestive Cardiac Failure (CCF) accounted for 18.75% cases, Cirrhosis of liver 13.40%, Tuberculosis 12.50% and Nephrotic Syndrome 9.00% cases. Out of the total 49 non-malignant transudative effusion, the distribution of causative diseases are as under:

**Table –3** Showing Causes of Non-Malignant Transudative Effusion

S.No	Causative Diseases	No. of Cases	Percentage
1	Congestive Cardiac Failure	21	42.85%
2	Cirrhosis of Liver	15	30.61%
3	Nephrotic Syndrome	10	20.40%
4	Pneumothorax	03	6.12%
	<b>Total</b>	<b>49</b>	<b>100.00%</b>

CCF was seen more commonly in females, whereas Cirrhosis of Liver and Nephrotic Syndrome seen more frequent in males.

All the Transudative Serous Effusions had protein levels less than 3 g%. Most cases of Transudative

Effusions seen were that of congestive Cardiac Failure and Cirrhosis. Predominant cells in these transudative Effusions were Lymphocytes.

**Table-4** Showing Total and Differential Counts of transudative non-malignant effusions along-with biochemical features.

Causative Disease	No. of samples	TLC Cells/Cu.mm			Predominant Cells				Proteins (gm%)	
		>500	500-1000	>1000	N	L	E	M	<3	>3
Congestive Cardiac Failure	21	17	04	-	04	17	-	-	21	-
Cirrhosis	15	07	08	-	01	14	-	-	15	-
Nephrotic Syndrome	10	10	-	-	10	-	-	-	10	-
Pneumothorax	03	03	-	-	03	-	-	-	03	-

N= Neutrophil L= Lymphocyte, E= Eosinophil, M=Monocyte

Out of 63 non-Malignant Exudative Effusions, Tuberculosis (22.22%) was the most common disease followed by pneumonia and Peritonitis

(each 14.28%), renal failure(12.70%) and COPD(7.93%)

**Table-5** Showing Causes of Non-Malignant Exudative Effusion

S.No	Clinical Feature	No. of Patients	Percentage
1.	Tuberculosis	14	22.22%
2.	Pneumonia	9	14.28%
3.	Peritonitis	9	14.28%
4.	Renal Failure	8	12.70%
5.	COPD	5	7.93%
6.	Acute Cholecystitis	4	6.34%
7.	Appendicitis	3	4.75%
8.	Hepatic Abscess	3	4.75%
9.	Pancreatitis	3	4.75%
10.	Empyema	3	4.75%
11.	Pelvic Abscess	2	3.17%
	<b>Total</b>	<b>63</b>	<b>100.00%</b>

**Table 6** Showing Total and Differential Counts and biochemical features of non malignant exudative

Causative Disease	No. of Samples	TLC Cells/Cu.mm			Predominant Cells				Protein (gm%age)
		0-500	500-1000	>1000	N	L	E	M	
Tuberculosis	14	06	-	-	01	13	-	-	14
Pneumonia	09	-	03	06	07	02	-	-	09
Peritonitis	09	-	09	-	09	-	-	-	09
Renal failure	08	-	06	02	06	02	-	-	08
COPD	05	02	03	-	03	02	-	-	05
Acute Cholecystitis	04	-	04	-	04	-	-	-	04
Appendicitis	03	02	-	01	03	-	-	-	03
Hepatic abscess	03	-	03	-	03	-	-	-	03
Pancreatitis	03	-	03	-	03	-	-	-	03
Empyema	03	-	02	01	03	-	-	-	03
Pelvic abscess	02	-	01	01	02	-	-	-	02

N=Neutrophil, L=Lymphocyte, E=Eosinophil, M=Monocyte.

From the above table, it is observed that lymphocytes were predominant cells in tuberculous exudates, where neutrophils predominated in other conditions.

Out of 28 malignant serous fluids, 23 (82.14%) were typed as adenocarcinoma, 2 (7.14%) were

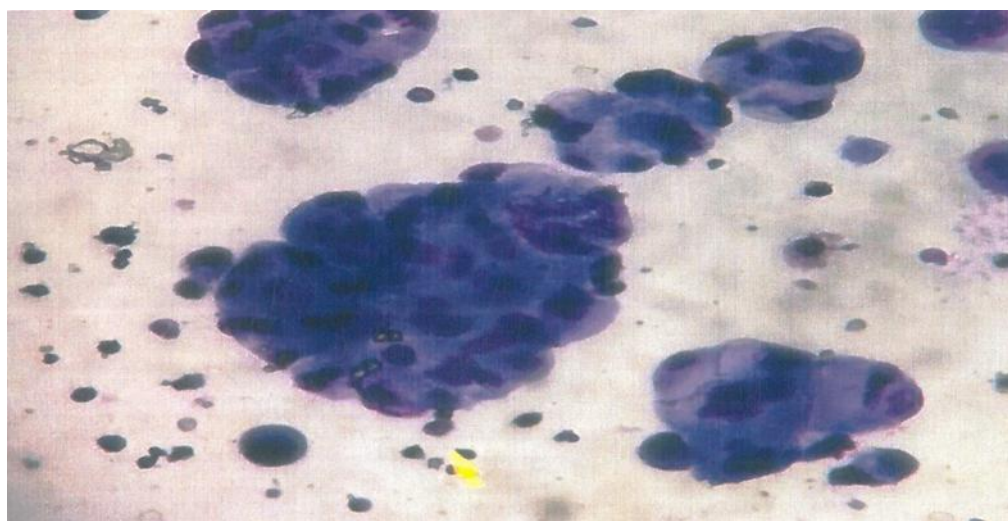
squamous cell carcinoma, 2 (7.14%) lymphoma and 1 (3.57%) was that of hepatocellular carcinoma. Out of 23 cases of adenocarcinoma, 13 were of pleural origin and 10 of peritoneal

**Table 7** Showing Primary Site and Sex Incidence of Malignant Effusions

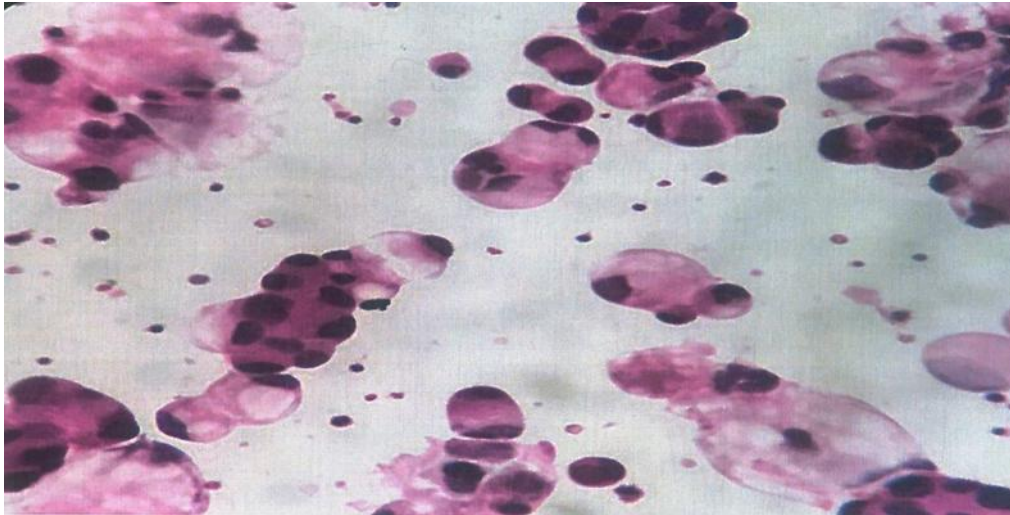
Site	Total no. of effusions	Pleural		Peritoneal	
		Male	Female	Male	Female
Carcinoma GIT	9	3	0	5	1
Primary carcinoma lung	8	5	3	0	0
Carcinoma ovary	4	0	1	0	3
Carcinoma breast	4	0	3	0	1
Lymphoma	2	0	0	1	1
Hepatocellular carcinoma	1	0	1	0	0
<b>Total</b>	<b>28</b>	<b>8</b>	<b>8</b>	<b>6</b>	<b>6</b>

The above table shows that amongst metastases in pleural effusions, most common primary site was lung & GIT in males; lung and breast in females. In peritoneal effusions, most common primary site was GIT in males; ovary, breast and GIT in

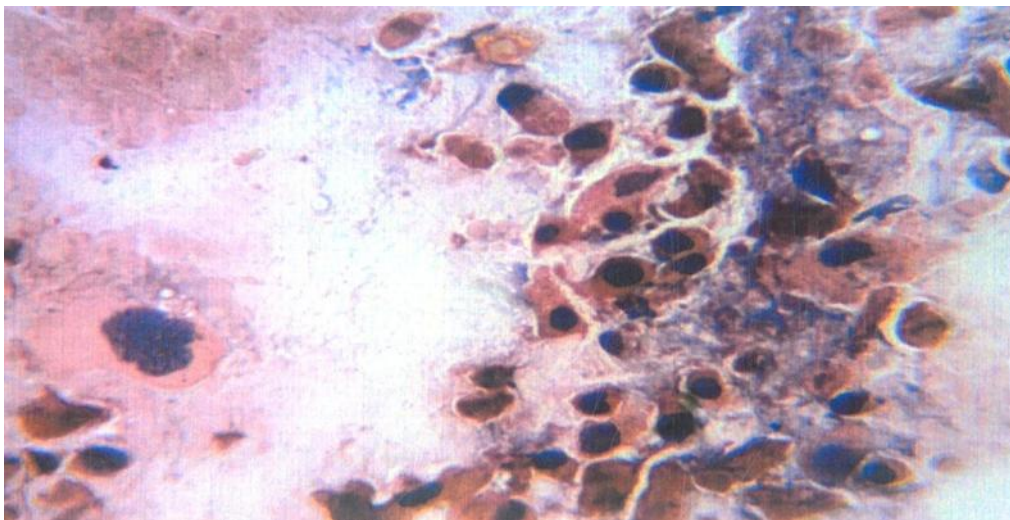
females. Two patients showed malignant lymphoma presenting as ascitis; one male and one female. One female patient presented with pleural effusion was diagnosed hepatocellular carcinoma.



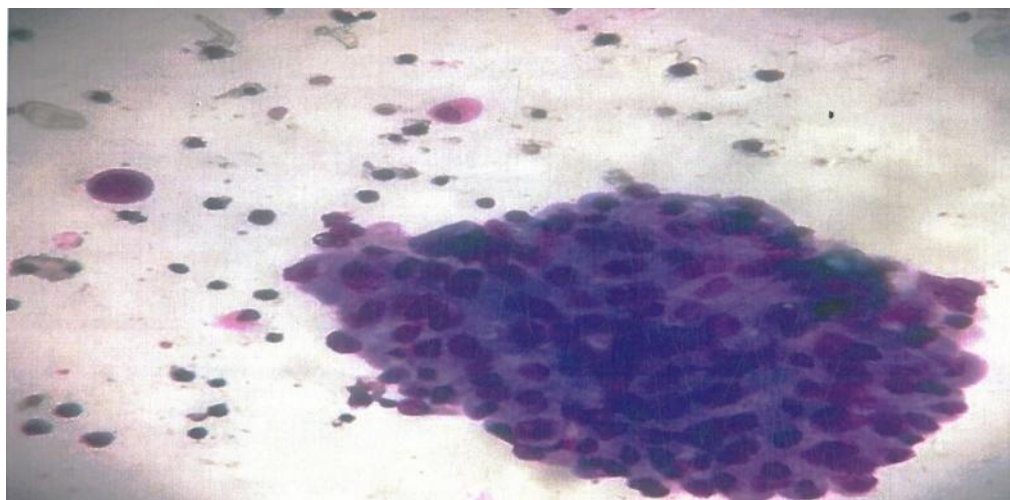
**High Power View showing adenocarcinoma with large tumor cells dense nuclei and Palecytoplasm (MGG x 400)**



Various Signet Ring Cell Seen (H&E x 400)



Marked Individual Cell Keratinisation Seen (PAP x 400)



Cells arranged in cohesive, ball like cluster with smooth border in metastatic breast carcinoma. Nucleus is moderately hyperchromatic and uniform in size (MGG x 400)

**Discussion**

The relative ease of pleural and ascitic fluid aspiration analysis and cytological examination

has kept alive the search for a test to unequivocally differentiate the various causes of effusion. The diagnostic performance of the

cytological study of fluid may be attributable to the fact that the cell population present is a representative of a much larger surface area than that obtained by needle biopsy<sup>1</sup>. Through the application of cellular diagnosis, a new phase of cancer study was brought into the realm of diagnosis and research. It is through the method of cytological smear that early detection of malignancy is performed<sup>6</sup>.

Cytological examination of serous fluids is of paramount importance because the finding of cancer cells in such a specimen denotes that the patient has cancer which is not only advanced but is also almost incurable and therefore, the cytological examination of serous effusions has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis often is considered the definitive test and obviates exploratory surgery. The present study deals with the accuracy of diagnosing on basis of contemporary cytological features cell count and biochemical features.

A total of 140 samples were studied. 73/140 (52.15%) cases were of pleural effusions and 67/140 (47.85%) were of peritoneal effusion. Male to female ratio in our study was 13:1 which is in accordance with the study done by Kumar et al (2000). The age range in our study was 7-80 years. Majority of effusions were noted above 40 years of age<sup>7</sup>.

In our study, 112/140 (80%) cases were non-malignant effusions and 28/140 (20%) were malignant effusions. Amongst non-malignant effusions, 57 (50.90%) cases were of pleural effusion, 55 (49.10%) of peritoneal effusions. This is in accordance with study of Thapar et al (2009) who in their study of a total of 120 cases of non-malignant effusions found the majority i.e 48.30% of cases as pleural effusion, followed by 45% of peritoneal effusion<sup>8</sup>.

Out of 57 non-malignant pleural effusions, 38 were of male patients and only 19 were from female patients. However, out of 55 non-malignant peritoneal effusions, 27 were of male and 28 of female patients. Among the 28 cases of

malignant effusions, 16 (57.15%) cases were of pleural effusion and 12 (42.85%) cases were of peritoneal effusion. Similar findings were recorded by Fiegl et al. (2004) who in their study got 55% cases of malignant pleural effusion and 45% cases of malignant peritoneal effusion<sup>9</sup>.

Out of total of 140 samples studied, 87 were exudative in nature and 53 transudative. Amongst 87 exudative cases, 63 were benign and 24 malignant. Out of 53 transudative effusions 49 were benign and only 4 were malignant. In our study the majority of fluids were exudative in nature which is not in accordance with the study done by Chung E S et al (1992) who reported the majority of the cases as transudative<sup>10</sup>.

The majority of non-malignant effusion cases comprised of 21 cases of CCF (18.75%), 15 cases of cirrhosis of liver (13.40%), 14 cases of tuberculosis (12.50%) and 10 cases of nephrotic syndrome (9%). Similar findings were recorded by Luse and Reagan (1954). In their study, majority of non-malignant effusions were due to CCF comprising 18% of all cases, followed by cirrhosis and tuberculosis comprising 11% and 8% cases respectively<sup>11</sup>.

The most frequent cause of exudative effusion was tuberculosis (22.22%), followed by malignancy (17.14%), pneumonia and peritonitis (14.28%). This is in accordance with the study done by Alusi (1986) and Kushwaha et al (2008)<sup>12,13</sup>. We had 14 cases of tuberculosis, 10 from pleural effusion and 4 from peritoneal. They all were exudative in nature. Lymphocytic predominance was seen in 13 cases and only 1 case showed neutrophilic predominance. Similar cytological findings of lymphocyte rich effusions in TB was reported by Malabu et al (2006)<sup>14</sup>. In our study 10 cases were from males and 4 from females.

Transudative effusions are usually characterised by a majority of lymphocytes. The pattern of predominant polymorphonuclear cells was seen in most effusions secondary to pneumonia, empyema, hepatic and pelvic abscess.

The most common primary site of malignant pleural effusion in males was lung and both lungs and breast in case of females. Di Bonito et al (1993) in his study found 77/143 cases of adenocarcinoma lung with pleural effusion<sup>15</sup>.

In peritoneal effusions, the most common primary site for males was GIT and for females it was ovary.

Adenocarcinoma formed the largest group of malignancy (82.14%) followed by squamous cell carcinoma (7.14%) and lymphoma (3.57%). Sears and Hajdu (1987) as well as Eid et al (2002) reported adenocarcinoma as commonest malignancy in their study (66% & 58%) respectively<sup>16,17</sup>.

We had 2 cases of squamous cell carcinoma. 1 male patient of 73 years old and another female of 58 years of age. Both cases were exudative in nature and presented as pleural effusion. In a study done by Ebru Cakir et al (2011), 2.7% of malignant pleural effusions contained atypical squamous cells<sup>18</sup>. Since squamous cell carcinoma is amenable to chemotherapy and radio therapy, it is important to recognise squamous carcinoma in patients to avoid unnecessary investigation and surgery.

NHL was diagnosed in 2 cases of peritoneal effusions. one was 75 years old male and another 70 years female. Both fluids were exudative in nature. Awasthi A et al (2007) in a study reported that pleural effusion is a relatively common finding in patients with NHL with the frequency of upto 20% and that malignant lymphocytes in effusions are usually identical to the cells in the involved lymph node and in blood<sup>19</sup>.

We had 3 cases of metastatic pleural effusion and 1 case of metastatic peritoneal effusion from breast carcinoma. Spieler et al (1985) confirmed similar findings in his study<sup>20</sup>.

We had 3 cases of metastatic peritoneal and 1 case of metastatic pleural effusion from primary in the ovary. Two were diagnosed as metastatic mucin secreting adenocarcinoma and other two were diagnosed as metastatic papillary carcinoma. S. Yoshimura M.D. et al (1984) in their study

confirmed that fluids that were positive for malignant cells were associated with serous and endometrioid carcinomas more often than with carcinomas of other types and also that patients with high stage tumors of all types had positive fluids more often than those with low stage tumors. The presence of tumor cells in the fluid indicated a worse prognosis at two years<sup>21</sup>.

In our study we found both Papanicolaou and Giemsa techniques to be complimentary. In Giemsa stained smears one could easily pick out the atypical cells due to their large size, and at the same time Papanicolaou stained smear was necessary to study the nuclear morphology.

### Conclusion

Hence we concluded that the most useful test in establishing the diagnoses of pleural and peritoneal effusion is their cytology and cell count and therefore forms a complete diagnostic modality which aims at pointing out the etiology of effusions and in others a means of prognostication of the disease process. Further studies need to be carried out including large number of cases along with application of newer technologies in the evaluation of effusions regarding etiology and in case of malignant effusions to determine the primary site. In the recent years with the availability of several antibodies, use of immunohistochemistry (IHC) and even molecular genetics on these fluid specimens, accurate diagnosis can be achieved and the typing of malignant cells has become more reliable.

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