Evaluation of Coelomic Fluids and its Clinical Correlation with Cytologic Diagnosis
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Abstract

Background: Aspiration of coelomic fluids from coelomic cavities is a simple and relatively, non-invasive technique to achieve a diagnosis. Pleural effusion, pericardial effusion and peritoneal effusion are commonly encountered problem by general physicians and chest physician. Effusion often present as a common diagnostic dilemma as no cause can be found in many cases inspite of careful evaluation. A better knowledge of spectrum of clinical history and clinical signs of pleural effusion, pericardial effusion and peritoneal effusion along with radiological, biochemical and cytological evaluation of the fluids helps in narrowing the diagnostic dilemma faced by physicians and helps in better management of patients.

Objective: This study was undertaken to evaluate the coelomic fluids and its clinical correlation with cytologic diagnosis.

Method: This was a prospective study conducted on 70 patients of pleural, pericardial and peritoneal effusions, satisfying inclusion criteria during the period between January 2012 to May 2013, presenting to the department of pathology, College of medical Sciences-Teaching hospital, Bharatpur. A detailed clinical history of the patient was obtained from the clinical protocol of hospital records in the proforma. Fluid from the pleural, pericardial and peritoneal cavity was obtained by thoracocentesis, pericardiacentesis and paracentesis performed by clinicians and analyzed for cytological diagnosis. Then, the cytological findings and clinical diagnosis were correlated. Statistical analysis was done using SPSS (Statistical Package for Social Sciences). The body fluids submitted from different department of COMS-TH were evaluated by cytological study and its clinical correlation was done. The prime objective of this study was to convey a diagnosis which would guide a clinician to correct diagnosis, hence the therapy.

Results: The present study includes 70 cases of pleural fluid, pericardial fluid and peritoneal fluid and analysis was done. Out of 70 cases, 34 cases are of peritoneal fluid, 31 cases are of pleural fluid and five cases are of pericardial fluid. Age of the patients ranged from eight years to ninety years with males to female ratio of 1.6:1 with a mean age of 50.36 years. The most common presenting complain were abdominal distension, swelling of lower limbs and yellowish discoloration of sclera (42.9%) followed by cough, fever, chest pain, shortness of breath, loss of appetite and loss of weight (30%) followed by abdominal distension, swelling of lower limbs, yellowish discoloration of sclera, loss of appetite and loss of weight (12.9%) followed by chest pain, shortness of breath, loss of appetite and loss of weight (7.1%) followed by cough, fever, and chest pain (5.7%) followed by loss of appetite and loss of weight (1.4%).

Out of 70 cases, 59 cases diagnosed as chronic effusion (84.28%). Six cases are diagnosed as inflammatory effusion (8.57%) and five cases are of malignant effusion (7.14%) on cytological evaluation. Out of five cases of malignant effusion two cases are of malignant mesothelioma. Of the total 70 cases of effusion, 50% were turbid, 47% were clear and 2.9% were hemorrhagic in appearance. 52.9% of effusions were exudative and 47.1% of effusion were transudative. The mean fluid glucose level on transudative effusion were in the range of 80.15 ± 47% were clear and 2.9% were hemorrhagic in appearance. 52.9% of effusions were exudative and 47.1% of effusion were transudative. The mean fluid glucose level on transudative effusion (8.57%) and five cases are of malignant effusion (7.14%) on cytological evaluation. Out of seven cases of pleural fluid, pericardial fluid and peritoneal fluid and analysis was done. Out of 70 cases, 34 cases are of peritoneal fluid, 31 cases are of pleural fluid and five cases are of pericardial fluid. Age of the patients ranged from eight years to ninety years with males to female ratio of 1.6:1 with a mean age of 50.36 years. The most common presenting complaint were abdominal distension, swelling of lower limbs and yellowish discoloration of sclera (45.9%) followed by cough, fever, chest pain, shortness of breath, loss of appetite and loss of weight (30%) followed by abdominal distension, swelling of lower limbs, yellowish discoloration of sclera, loss of appetite and loss of weight (12.9%) followed by chest pain, shortness of breath, loss of appetite and loss of weight (7.1%) followed by cough, fever, and chest pain (5.7%) followed by loss of appetite and loss of weight (1.4%).

Out of 70 cases, 59 cases diagnosed as chronic effusion (84.28%). Six cases are diagnosed as inflammatory effusion (8.57%) and five cases are of malignant effusion (7.14%) on cytological evaluation. Out of five cases of malignant effusion two cases are of malignant mesothelioma. Of the total 70 cases of effusion, 50% were turbid, 47% were clear and 2.9% were hemorrhagic in appearance. 52.9% of effusions were exudative and 47.1% of effusion were transudative. The mean fluid glucose level on transudative effusion were in the range of 80.15 ± 21.19 mg/dl which was higher as compared to exudative effusion and this difference was statistically highly significant (p value = 0.010). The mean fluid total protein levels in transudative effusion were in the range of 2.08 ± 0.43 gm/dl which can be classified as transudative form as compared to exudative form and this is highly statistically significant (p value = 0.010). The average cell count in malignant effusion, chronic effusion and inflammatory effusion were 620 cells/mm3, 520 cells/mm3 and 31,141 cells/mm3 respectively.

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Received October 19, 2015; Accepted February 05, 2016; Published February 08, 2016.


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Lymphocytes were predominantly seen in patients with chronic effusion. Lymphocytes were also seen in cases of malignant effusion. Five patients with malignant effusion showed malignant cells in fluid cytology. The estimated mean ± standard deviation of all the transudative fluid total leukocyte count were 188.57 ± 53.65 and of exudative fluid cell count were 5793.29 ± 24576.28. This was statically not significant (p value = 0.141 using chi-square test). After comparison of results of cytology with clinical diagnosis overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value (PPV) was 60% and negative predictive value was 96.92%. The study was statistically significant (p value = 0.003).

Conclusion: The most useful test in establishing the diagnosis of pleural, pericardial and peritoneal effusion is pleural, pericardial, and peritoneal fluids cytology and fluid cell count. Cytological study of the fluid is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as, in certain cases, a means of prognostication of disease process. Although many diseases are known to cause pleural, pericardial and peritoneal effusions, analysis of the fluid, in conjunction with clinical acumen, pinpoints the etiology in the majority of cases. It was observed in study that cytology had higher sensitivity, specificity and accuracy in the diagnosis of malignant effusions.

However, there are some difficulties and limitation in the diagnosis with cytology alone, in both false-negative and false-positive results can occur. The other drawback of fluid cytology is the patients with probable tuberculosis were not followed up without treatment. All the suspected cases of effusion should undergo ultrasonography along with chest x-ray and fluid cytology should be done to confirm tuberculosis or to rule out malignancy which guides the physicians for further evaluation of the patient if required. Routine analysis of pleural, pericardial and peritoneal fluid total protein, glucose and LDH helps to differentiate between transudate and exudate. It also helps to differentiate between tubercular and non-tubercular effusion. Poor survival among cancer patients with pericardial effusion and abnormal fluid cytology may have important implications for management.

**Keywords:** Exudate; Transudate; Tubercular; Malignant; Empyema; Pleural effusion; Pericardial effusion; Peritoneal effusion; Accuracy; Sensitivity; Specificity

### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>CCF</td>
<td>Congestive Cardiac Failure</td>
</tr>
<tr>
<td>COMS-TH</td>
<td>College of Medical Sciences-Teaching Hospital</td>
</tr>
<tr>
<td>CXR</td>
<td>Chest X-ray</td>
</tr>
<tr>
<td>DPX</td>
<td>Distrene Dibutyl Phthalate Xylene</td>
</tr>
<tr>
<td>e.g.</td>
<td>Example</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
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<td>Edn.</td>
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</tr>
<tr>
<td>et al.</td>
<td>And Others</td>
</tr>
<tr>
<td>FN</td>
<td>False Negative</td>
</tr>
<tr>
<td>FP</td>
<td>False Positive</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>L</td>
<td>Lymphocyte</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
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<tr>
<td>LPE</td>
<td>Left Sided Pleural Effusion</td>
</tr>
<tr>
<td>MGG</td>
<td>May Grunwald's Giemsa Stain</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MS</td>
<td>Microsoft</td>
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<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
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<td>P</td>
<td>Polymorphs</td>
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<tr>
<td>PAP</td>
<td>Papanicolaou Stain</td>
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<tr>
<td>PAS</td>
<td>Periodic acid-Schiff</td>
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<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
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<tr>
<td>RPE</td>
<td>Right Sided Pleural Effusion</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>TLC</td>
<td>Total Leukocyte Count</td>
</tr>
<tr>
<td>TN</td>
<td>True Negative</td>
</tr>
<tr>
<td>TP</td>
<td>True Positive</td>
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<tr>
<td>USG</td>
<td>Ultrasonography</td>
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<tr>
<td>XR</td>
<td>X-ray</td>
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### Introduction

Abnormal accumulation of fluid in the pleural cavity is known as pleural effusion. Diagnosis of pleural effusion requires a proper clinical evaluation and cytological study. Aspiration of pleural fluid is a simple and relatively non-invasive technique. Pleural fluid analysis and cytology remains the main stay for diagnosing the various diseases [1]. Pleural fluid analysis can allow the follow up of the patient after treatment [2]. Approximately one million patients develop pleural effusion every year [3]. Thoracocentesis can be safely performed to collect the pleural fluid [4]. Both malignant and non-malignant cases of effusion can be identified by relative technique of pleural fluid cytology [5]. But some studies revealed pleural fluid cytology was not helpful in ascertaining few cases of pleural effusion of patients, there by indicating its limitation [5,6]. Ascites, defined as fluid accumulation in the peritoneal cavity, may accompany several diseases. Patients who suffer from ascites present a diagnostic and therapeutic problem [7,8]. Biochemical analysis of pleural, pericardial and peritoneal fluid is very useful tool to assess the etiology, pathophysiology and subsequent treatment of effusion [9]. Pericardial fluid was obtained by pericardiocentesis for the medical management of underlying disease [10]. The careful cytomorphological examination of pericardial fluid aspirates is a valuable, reliable, and diagnostically highly accurate method, which could be performed on a routine basis in a busy cytopathology department. Judiciously chosen ancillary procedures, as well as clinico-pathological correlation, are of great value for an accurate diagnosis in problematic cases [11].
Review of Literature

Historical aspects

Pleural effusion [12] was first described by Hippocrates as early as in 5th century B.C. in a patient with pneumonia. Hippocrates also used the term ‘pleurisy’ but he used this term to refer to the sidewall of thoracic cage rather than the lining membrane. Hippocrates referred to the disease associated with cough, fever, blood spitting and emaciation as ‘phthisis’ and believed that it was due to the ulceration of lung [12]. Later in 1820, Laennec described pneumothorax and hemorrhagic pleurisy and the association of phthisis and pleurisy with effusion. Armand Trouseau in the 18th century, an eminent physician from Paris, aspirated fluid from pleural cavity, for the first time. This procedure was improved by his pupil Georges Dieulafoy who used a trocar in the aspiration of the pleural fluid. Defrancis and associates used a Vim Silvermann needle for the first time for obtaining biopsies from the parietal pleura. Abrahamus performed a pleural biopsy using a Harrifield biopsy needle. Emerson described pleural effusion due to lymphatic obstruction (yellow Nail syndrome). Gaensler and Kaplan described benign pleural effusions due to exposure to asbestosis. Light and Ball described the measurement of pleural fluid lactic dehydrogenase to differentiate transudates from exudates. The cytologic study of body fluids is one of the oldest applications of cytologic technique, first investigated in the later half of 19th century. The purpose is to determine the cause of fluid accumulation in body cavities such as the pleura, pericardium, and the abdominal cavity (ascitic fluid) [13].

Ascitic fluid history [12] starts since the 1500 B.C. when Egyptians were aware of abnormal collection of abdominal fluid associated with the disease of liver. Celsus (20 B.C.) advocated removal of fluid to give relief to the patients with abdominal swelling. In 700 A.D. Paul of aegina treated abdominal swelling by draining through a copper tube while the term analysis started in 1950 when Keith and Pfeffer noted raised amylase levels in peritoneal fluid in the case of Pancreatitis. Geigs B and Kunkel measured osmotic pressure of ‘Ascites’ was coined later in 1398 by Trevesia. Laennec described peritonitis. James Rutherford Morrison introduced ‘omentopexy’ to relieve ascites due to cirrhosis of liver [12].

Anatomy and physiology related to pleural fluid

Pleural fluid formation takes place because of increased formation or decreased absorption or both [4,15].

Increased pleural fluid formation: The various mechanisms by which increased pleural fluid formation occurs are:

• Increased interstitial fluid formation

It occurs when the amount of fluid exceeds 5 g per gram of lung dry weight; pleural fluid accumulates whether the edema is high protein or low protein. This occurs in:

a. Congestive cardiac failure
b. Para pneumonic effusion
c. Acute respiratory distress syndrome
d. Lung transplantation

Increased hydrostatic pressure gradient

If there is an increased pressure gradient between the intravascular pressure and pleural pressure there will be an increase in the rate of pleural fluid formation. This occurs in:

a. Right ventricular failure
b. Left ventricular failure
c. Superior venacaval obstruction
d. Increased capillary permeability

Inflammation of the pleural surface causes increase in the capillary permeability leading to pleural fluid formation.

• Decreased oncotic pressure gradient

Decreased oncotic pressure gradient leads to the formation of pleural fluid through its influence on the Starling’s equation as in hypoproteinemic states.

• Disruption of the thoracic duct or intra-thoracic blood vessel

Trauma to the thoracic duct and intercostal vessels can lead to formation of chyle or blood in the thoracic cavity respectively.

Decreased pleural fluid absorption

• Lymphatic obstruction

Lymphatics are responsible for majority of pleural fluid absorption from the pleural space. Obstruction to lymphatics is most commonly seen in malignant effusion where the lymphatics are obstructed by malignant cells.

• Elevation of systemic venous pressure

As venous pressure increases, the pleural fluid accumulation increases exponentially as in superior venacaval obstruction.

Anatomy and physiology related to peritoneal fluid

Peritoneum is an alveolar membrane covered by single layer of mesothelial cells. The peritoneum is divided into parietal and visceral components. The parietal peritoneum covers the anterior, lateral and posterior abdominal walls, the inferior surface of diaphragm, and the pelvis. The surface of the intra-peritoneal organ is covered by visceral peritoneum whereas only the anterior aspect of the retroperitoneal organs is covered by visceral peritoneum. The peritoneal cavity is a
complicated potential space that is between the visceral and parietal peritoneum and is normally empty except for a few microliters of peritoneal fluid. The spread of infection within the peritoneal cavity is governed by the site of infection, the location of fibrinous adhesions, intra-peritoneal pressure gradient, and the position of the patient. Visceral peritoneum is supplied by the splanchnic blood vessels and the parietal peritoneum is supplied by intercostal, subcostal, lumbar and iliac vessels. Similarly visceral peritoneum is supplied by visceral non-somatic sympathetic nerves and the parietal peritoneum is supplied by somatic nerves. Fluid, solutes and particulate matter appear to be absorbed by different routes from the peritoneal cavities [17].

**Anatomy and physiology related to pericardial fluid**

The heart is enclosed in a sac-like structure called the pericardial sac, which is lined by mesothelial cells. The lining covering the outside of the heart is the visceral pericardium and the lining covering the inside of the pericardial sac is the parietal pericardium. During certain pathologic event, fluid may accumulate in the pericardial sac. If the fluid accumulates rapidly (a minimum of 250 ml) or if a relatively large amount (1000 ml) accumulates over a longer period of time, there may be a serious restriction to the normal heartbeat, creating a life threatening event. This is called cardiac tamponade [18]. Pericardial fluid is an ultra-filtrate of plasma, normally measuring 15-35 ml possibly with some overflow of lymph drainage and myocardial interstitial fluid [19].

The pericardial cavity is only a potential cavity formed by two serous membranes that are closely opposed to each other and separated by a small amount of serous fluid. This fluid allows the heart to move easily during contraction and relaxation. The accumulation of fluid within the pericardial cavity is called pericardial effusion [9]. The pericardial cavity normally contains 10-20 ml of pericardial fluid secreted by the pericardial membranes [20]. There is a continuous production and resorption of pericardial fluid [21]. The mesothelial cells of the parietal pericardium have extensive microvilli that facilitate the exchange of fluid and small molecules [22].

Histology of the pleural, the peritoneal and pericardial cavities: The three cavities, generally designated as body cavities, have a common embryologic origin in the mesenchymal embryonal layer. The pleura encloses the lungs, peritoneum, the intestinal tract, and the pericardium, the heart. The body cavities are lined by a single layer of flat cells—the mesothelium, supported by the connective tissues and an appropriate, the vascular and nervous apparatus [23]. The parietal and visceral pleurae are developed from somatopleural and splanchnopleural layers of the lateral plate of mesoderm respectively. [14] (Figure 1 and Table 1).

**Etiology of pleural effusion**

Effusion refers to the accumulation of abnormal amount of fluid in the pleural space [4,15,24-26]. It may be due to number of factors like:

- Diseases primarily involving the pleura
- Diseases of the lung involving the visceral pleura
- Due to factors causing generalized edema

The first step in diagnosing a pleural effusion is to determine whether the fluid is a transudate or exudate. These two types of effusions are caused by different mechanisms and identifying the nature of the effusion will help in diagnosing the underlying disorder. In some cases, the etiology of effusion is obvious from the clinical picture (e.g. Bilateral pleural effusions in congestive heart failure) (Table 2).

**Transudative effusion**

Transudative effusions occur when mechanical factors influencing the formation or reabsorption of pleural fluid are altered [4,15,24-26]. This results in the flow of low protein into the pleural space. The characteristics of transudative effusions are:

- Transudates are clear thin fluids
- Transudates are often bilateral
- Specific gravity is less than 1.015
- The amount of protein is below 3 gm%
- On cytological analysis, transudates have a lower leukocyte count-more than 80% transudates have a WBC count of less than 1000 mm³ with only a few lymphocytes

- The culture is sterile
- The fluid does not clot on standing

Causes of transudative pleural effusions are:

a. Increased hydrostatic pressure
   - Congestive cardiac failure
   - Constrictive pericarditis
   - Pericardial effusion
   - Constrictive cardiomyopathy
   - Massive pulmonary embolism

b. Decreased capillary osmotic pressure
   - Cirrhosis of liver
   - Nephrotic syndrome
   - Malnutrition
   - Protein losing enteropathy
   - Small bowel disease

c. Transmission from peritoneum
   - Any cause of ascites
   - Peritoneal dialysis

d. Increased capillary permeability
   - Small pulmonary emboli
   - Myxedema

e. Miscellaneous
   - Acute atelectasis
   - Wet Beriberi
   - Idiopathic

**Exudative effusion**

Exudative effusions are caused by alterations in the capillary permeability due to disease process affecting the pleura like inflammation, injury or neoplasm and hence contain a higher concentration of proteins [4,15,24-26]. Exudative effusions have following characteristics:

- Fungal and actinomycotic disease
- Viral infections
- Sub-diaphragmatic abscess
- Hepatic amoebiasis
- Parasitic infection

b. Neoplasms
- Bronchogenic carcinoma
- Metastatic disease
- Pulmonary secondaries
- Mesothelioma of pleura
- Lymphoma
- Leukemia
- Pleural sarcoma
- Chest wall tumors
d. Collagen vascular disease
- Post myocardial infarct
- Rheumatoid disease
- Systemic lupus erythematosus
- Rheumatic fever
- Drug induced lupus
- Sjogren’s syndrome
- Churg-Strauss syndrome
- Immunoblastic lymphadenopathy
- Sarcoidosis
e. Gastrointestinal disease
- Acute pancreatitis
- Esophageal perforation
- Diaphragmatic hernia
- After abdominal surgery
- After liver transplant
- Malignant mesothelioma of peritoneum
- Hepatic abscess
- Psuedomyxoma peritoneii
f. Pulmonary embolism and infarction
g. Miscellaneous
- Meigs syndrome
- Drug reactions
- Radiation therapy
- Asbestos exposure
- Recurrent polyserositis

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of cases</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>21-40</td>
<td>9</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>41-60</td>
<td>20</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>61-80</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>81-100</td>
<td>3</td>
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<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>43 (61.43%)</td>
<td>27 (38.57%)</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 1: Age and sex distribution of patients.

- Exudates are opalescent or turbid fluids. They are sometimes purulent or mucopurulent
- They are usually unilateral
- The specific gravity is more than 1.015
- The level of proteins is more than 3 gm%
- Exudates have a higher leukocyte count with an increased number of lymphocytes or polymorphs
- Culture may yield an organism
- The fluid often clots on standing

Causes of exudative effusions are:

a. Infections
- Bacterial infections
- Lung abscess
- Pulmonary tuberculosis
Clinical features of pleural effusion

The clinical features of pleural effusion depend on the amount, rate of accumulation of fluid and the underlying disease [12,15,27]. Large pleural effusions or effusions where the fluid accumulation occurs rapidly produce acute symptoms. Small effusions or even large effusions where the fluid accumulation occurs very slowly result in little or no symptoms. Thus, the onset of symptoms, depending on the nature of the pleural effusions may be acute, sub-acute or insidious. In acute cases, the symptoms appear suddenly and will be severe in nature whereas in the sub-acute and insidious onset, a gradual progression is noticed with a varying periods of ill-health and prodromal symptoms. Symptoms of pleural effusions are as follows:

- Pleuritic chest pain
- Dyspnoea
- Cough
- Fever
- Weight loss, anorexia and lassitude

Physical signs due to accumulation of fluid in pleural space are as follows:

- Respiratory movements may be reduced or absent on the affected side
- A fullness or prominence of the chest wall on the affected side may be seen
- Mediastinal displacement to the opposite side will be seen
- Stony dullness on percussion
- Decreased or absent breath sounds on auscultation
- Pleural rub may be heard

Investigations in pleural effusion

Radiological investigations:

1. X-Ray Chest
   - An effusion is visible radiographically as a homogenous opacity [27]
   - An elevation of hemidiaphragm
   - Collapse of the lung

2. Ultrasonography
   - Sonography can be used to differentiate pleural from parenchymal lesions to visualize disease parenchyma hidden by pleural effusion and other pleural abnormalities [27] (Table 3).
   - Pleural fluid that is anechoic is usually a transudate although occasionally anechoic fluid may prove to be exudates. Fluid that is echogenic contains floating particulate matter, septations or fibrin strands or an associated with pleural nodules or pleural thickening.

3. Computerized Tomography of the pleura
   - On high resolution computed tomography (CT) the pleura may be identified when it forms a fissure. A pleural effusion appears on CT as a dependent, sickle shaped opacity with lower CT number than that of any adjacent pleural thickening or mass. CT numbers do not allow a distinction between transudate and exudate, but parietal pleural thickening on contrast enhanced CT almost always indicates the presence of a pleural exudate.

4. Magnetic Resonance Imaging
   - Pleural fluid has a low signal on T1 weighted sequences and a high signal on T2 weighted images, with a tendency for exudates to give a higher signal than transudates on T2 weighted sequences [27] (Table 4).

Thoracocentesis: Thoracocentesis is the mainstay of diagnosis [25,27,28]. A diagnostic thoracocentesis is usually done when the cause of effusion is uncertain. The indications for thoracocentesis are:

1. For diagnostic purpose
   - Massive pleural effusions
   - Bilateral effusions

<table>
<thead>
<tr>
<th>Type of fluid</th>
<th>Number of cases</th>
<th>Cytologic diagnosis</th>
<th>Total</th>
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<tr>
<td></td>
<td>Malignant effusion</td>
<td>Chronic effusion</td>
<td>Inflammatory effusion</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>31</td>
<td>1 (3.2%)</td>
<td>24 (77.4%)</td>
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<tr>
<td>Pericardial fluid</td>
<td>5</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>34</td>
<td>2 (5.9%)</td>
<td>32 (94.1%)</td>
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<tr>
<td>Total</td>
<td>70</td>
<td>5</td>
<td>59</td>
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</table>

Table 3: Showing type of fluid and cytologic diagnosis.

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<th>Female</th>
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<td>21-40</td>
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<td>4</td>
<td>7</td>
<td>22.6%</td>
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<td>19.4%</td>
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<tr>
<td>81-100</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>9.7%</td>
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<tr>
<td>Total</td>
<td>18 (58.1%)</td>
<td>13 (41.9%)</td>
<td>31</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Thus out of 31 cases of pleural fluid, 18 were males (58.1%) and 13 were females (41.9%).

Table 4: Age and sex distribution of patients in pleural fluid.
Cardiac and respiratory embarrassment
Secondary infection or synpneumonic effusion
2. Therapeutic thoracocentesis

Usually large amount of fluid aspiration, but not more than 1000-1500 ml of fluid is aspirated at the initial attempt.

3. Biochemical analysis of pleural fluid [4,12,15,24,29]

i. Total protein

Transudates have a protein content of less than 3 gm/dl whereas exudates have a protein content of more than 3 gm/dl.

ii. Glucose and pH

The amount of glucose in pleural fluid transudate is normally more than 60 mg/dl. Transudate usually have a pH of 7.4-7.5 and exudates have a pH of 7.35-7.45.

iii. Amylase

Amylase levels of more than 1000 somogyi units are seen in pleural effusions associated with pancreatitis and esophageal rupture. Elevated pleural fluid amylase in the absence of pancreatitis is highly suggestive of malignancy.

iv. Lipids

Increased presence of lipids imparts a milky or opalescent color to the effusion and such an effusion that contains chyle is called chylothorax. Cholesterol levels are more than 60 mg/dl in case of exudates whereas transudates have a cholesterol levels below 60mg/dl.

v. Lactate dehydrogenase (LDH)

All exudative effusions show elevated LDH levels.

Cytological Analysis of Pleural Fluid

Cytology is concerned primarily with the morphological features of individual or small cluster of cells. The primary feature of diagnostic importance of pleural fluid cytology is the exclusion or confirmation of malignancy. In addition, the number and nature of inflammatory and other cells within the effusion may provide valuable clues concerning etiology [15,29-31] (Table 5).

Pleural fluid analysis should always include total cell count, and careful cytological examination to identify malignant cells. Normal pleural fluid contains about 1500 cell/mm³, with a predominance of monocytes and some lymphocytes, macrophages and mesothelial cells. Polymorphs are rare. A cell count of 90-95% lymphocyte is usually followed by chest pain, shortness of breath, loss of appetite and loss of weight (64.5%) followed by abdominal distension, swelling of lower limbs and yellowish discoloration of sclera (19.4%) followed by cough, fever, and chest pain (12.9%) followed by chest pain, shortness of breath, loss of appetite and loss of weight (3.2%).

Table 5: Distribution of presenting complain in patients of pleural fluid.

<table>
<thead>
<tr>
<th>Presenting complain</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough + fever + chest pain + shortness of breath + loss of appetite + loss of weight</td>
<td>20</td>
<td>64.5%</td>
</tr>
<tr>
<td>Abdominal distension + swelling of lower limbs + yellowish discoloration of sclera</td>
<td>6</td>
<td>19.4%</td>
</tr>
<tr>
<td>Cough + fever + chest pain</td>
<td>4</td>
<td>12.9%</td>
</tr>
<tr>
<td>Chest pain + shortness of breath + loss of appetite</td>
<td>1</td>
<td>3.2%</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 6: Showing the estimated mean ± SD of all fluid glucose and protein in transudative effusion and exudative effusion.
Due to increased hydrostatic pressure or decreased plasma oncotic pressure
1. Congestive cardiac failure
2. Hepatic cirrhosis
3. Hypoproteinemia
4. Nephrotic syndrome

Exudative causes:
- Increased capillary permeability or decreased lymphatic resorption
  1. Infections
     - Tuberculosis
     - Primary Bacterial Peritonitis
     - Secondary bacterial peritonitis (e.g. Appendicitis)
  2. Neoplasm
     - Hepatoma
     - Metastatic carcinoma
     - Lymphoma
     - Mesothelioma
  3. Trauma
     - Pancreatitis
     - Bile peritonitis (e.g. Ruptured gall bladder)
  4. Chylous Effusion

Clinical features of Ascites [39]
- Abdominal distension
- Abdominal fullness/swelling
- Lower limb swelling
- Fatigue
- Anorexia
- Bleeding manifestations- epistaxis, gum bleeding etc.
- Yellowish discoloration of sclera, urine history of alcohol use

Physical examination
- Poor nutritional status
- Muscle wasting
- Thin limbs with protuberant belly
- Edema in the lower limbs
- Cyanosis, spider telangiectasia, palmar erythema, flapping tremors
- Bruises, purpura, echymosis
- Hepatomegaly, splenomegaly
- Abdominal distension

Dilated superficial veins
- Shifting dullness and fluid thrill

Investigations
1. Abdominal paracentesis - fluid sent for evaluation [24,36,40]
   Ascitic fluid collected by abdominal paracentesis is examined under following headings

   A) Physical Examination
   1. Volume
   2. Color
   3. Appearance
   4. pH
   5. Specific gravity
   6. Clot formation

   B) Biochemical Examination
   1. Protein
   2. Amylase
   3. Lipase
   4. Sugar
   5. Ammonia
   6. Lactate dehydrogenase
   7. Albumin
   8. Alkaline phosphatase
   9. Adenosine deaminase
   10. Serum Ascites Albumin gradient

   C) Cytological Examination
   1. Red blood cell (RBC): These may be found in transudate in small number. RBCs in excess of in an exudative fluid may indicate a malignant process.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Cytologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic effusion</td>
</tr>
<tr>
<td>Cirrhosis of liver</td>
<td>6 (19.4%)</td>
</tr>
<tr>
<td>Pulmonary tuberculosis</td>
<td>15 (48.4%)</td>
</tr>
<tr>
<td>Empyema</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>3 (9.7%)</td>
</tr>
<tr>
<td>Carcinoma of lung</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (77.4%)</td>
</tr>
</tbody>
</table>

*Out of 31 cases of pleural fluid, fifteen cases are clinically diagnosed as pulmonary tuberculosis (48.4%), six cases are of cirrhosis of liver (19.4%), four cases are of pneumonia (12.9%), three cases are of congestive cardiac failure (9.7%), two cases are of empyema (6.5%) and one case of carcinoma of lung (3.2%). 24 cases are cytologically diagnosed as inflammatory effusion, six cases are diagnosed as chronic effusion and one case was diagnosed as malignant effusion. Out of 31 cases, 12 cases are exudates (38.7%) and eight cases are transudates (25.8%). Out of 31 cases, 22 cases are turbid in appearance (71.0%), eight cases are clear (25.8%) and one case was hemorrhagic (3.2%).

Table 7: Showing clinical and cytologic diagnosis in pleural fluid.
2. White blood cell (WBC): Presence of large number of neutrophils indicates acute bacterial inflammation. Presence of a large number of lymphocytes indicates a chronic inflammatory process. Presence of mixed population of inflammatory cell is often found in malignancy.


4. Mesothelial cells: Found in almost all types of pathological fluid mixed with either inflammatory cells or malignant cells. Sometimes difficult to differentiate from carcinoma cells. Mesothelial cells undergo hypertrophy and hyperplasia in response to a wide variety of stimuli. Protopytic mesothelial cells are round, have dense cytoplasm which tends to fade at the periphery, smoothly contoured, central or slightly eccentric nuclei smooth nuclear membrane and readily visible nucleoli.

5. Malignant cells: Malignant cells are found in carcinomatous infiltration. Malignant lymphoma cells are found in infiltration of mesothelium by lymphoma. Malignant cells in fluids can usually be distinguished as hematopoietic (leukemia, lymphoma) versus non hematopoietic (carcinoma, sarcoma). It is important to look at the entire cellular area of the slide with a low power objective to detect suspicious clusters of cells. General features of malignant cells include an irregular nuclear membrane, unevenly distributed chromatin, and nucleoli that also have irregular membrane.

D) Microbiological Examination

1. Gram stain
2. Ziehl-Neelsen (ZN) Stain II. Ultrasonography

Pericardial effusion

Pericardial effusion is the accumulation of more than the usual amount of fluid in the pericardial sac [41].

Causes of pericardial effusion [29,41,42]:

Exudates

- Infections (including HIV)
- Myocardial infarction
- Neoplasm
- Trauma
- Uremia
- Radiation therapy

Clinical features [29,41,42]:

Symptoms:

- Dyspnea
- Dull retrosternal ache
- Heart sounds are soft and distant
- Apex beat is commonly obscured
- Raised jugular venous pressure

Signs:

- Cardiac dullness beyond the apex
- Tachycardia
- Narrow pulse pressure
- Pulsus paradoxus
- Fever
- Low blood pressure

Investigations

1. ECG reveals low-voltage QRS complexes.
2. Chest x-ray shows large globular or pear shaped heart with sharp outlines with cardiomegaly.
3. Echocardiography is the most useful technique for demonstrating effusion.
4. MRI.
5. Pericardiocentesis: It is the removal of pericardial fluid with aseptic technique under echocardiographic guidance.
6. Pericardial biopsy: may be needed if tuberculosis.
7. Cytological analysis of pericardial fluid.

Materials and Methods

The present study was undertaken to study the cytology of pleural, pericardial and peritoneal fluid and compared them with clinical diagnosis were studied prospectively from January 2012 to May 2013 in the department of pathology, College of Medical Sciences-Teaching Hospital, Bharatpur, Nepal. A detailed clinical history of the patient was obtained from the clinical protocol of the hospital records in the proforma. Fluid from pleural, pericardial, and peritoneal cavity were obtained by thoracocentesis, pericardiocentesis and paracentesis performed by the clinicians and analyzed for cytological diagnosis. The samples were processed fresh but if delay was anticipated then the fluid was anticoagulated in EDTA (1mg per 1ml of fluid) and fixed by adding of equal volume of 50% ethyl alcohol and processed when convenient. If the fluid is hemorrhagic then 2% glacial acetic acid was used as a hemolysing agent which also exentuates nuclear staining. A cell count was performed with the help of a Neubauer chamber. The fluid was centrifuged for ten minutes at 2000 rpm (revolution per minute). The sediment was transferred to a clean grease free glass slide and evenly spread as a smear. Smears so prepared was stained with Giemsa stain (being routinely used in this department). Such stained smears were examined microscopically at 10X and 40X. If required, stains like Hematoxylin and Eosin (H and E), Papanicolaou (PAP) stain and other special stains like Mucicarmine and Periodic acid-Schiff (PAS) stains were used. If indicated by clinical features and routine cytological examination unstained smears were also used for identification of Acid Fast Bacilli by Ziehl-Neelsen (ZN) stain. All such stained smears were mounted with a cover slip using Distrene Dibutyl Phthalate Xylene (DPX) for filing/storage. If the amount of fluid was less than 1.0 ml then it was subjected to a cytospin smear preparation. Then clinical diagnosis and cytologic findings are correlated. (Figures 2 and 3) (Table 8).

Sample size: Prospective, Consecutive 70 cases

Duration of the study: January 2012 to May 2013

Place of study: Department of Pathology, College of Medical Sciences-Teaching Hospital, Bharatpur, Chitwan
Type of study: Descriptive type

**INCLUSION CRITERIA**

Only the body fluids (pleural, pericardial, and peritoneal fluids) are included in this study (Figures 4 and 5).

**Exclusion criteria**

- Cerebrospinal fluid (CSF) and synovial fluids were excluded from the study.
- Inadequate smears and fluid samples received late without above precautions were excluded from the study.

**Data entry and statistical analysis**

- Data from the patient were collected in the proforma designed for the study, attached with dissertation.
- Data entered in MS Excel File.
- Data analyzing using Statistical Package For the Social Sciences (SPSS) 16.0 software.
- For the correlation of cytological findings with clinical diagnosis, statistical analysis was done.

A true positive (TP) cytological result was defined as a neoplastic diagnosis determined to be neoplastic in clinical diagnosis. A false positive (FP) was defined as malignant cytologic diagnosis determined to be non-neoplastic on clinical diagnosis. A true negative (TN) was defined as benign (inflammatory effusion and chronic effusion) cytologic result determined to be non-neoplastic infection) in clinical diagnosis. A false negative (FN) was defined as a non-neoplastic diagnosis determined to be malignant in clinical diagnosis.

A test of significance was done by X-test (Chi-square test). The data

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of cases</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>0-20</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>21-40</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>41-60</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>61-80</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>81-100</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
</tbody>
</table>

*Out of 70 cases, 34 cases are of peritoneal fluid. Out of 34 cases 21 are males (61.8%) and 13 cases are females (38.2%). Ages of the patient with maximum cases in the age group of 41 to 60 years (58.8%).

**Table 8:** Showing age and sex distribution of the patient in peritoneal fluid.
was collected purposive sampling method and analyzed for frequency, percentage, specificity, sensitivity, predictive value of negative test and positive value of positive test. From above data p value was calculated which was the basis for the interpretation of the study (Table 9).

**Formula**

- Sensitivity = \( \frac{a}{a+c} \times 100 \)
- Specificity = \( \frac{d}{b+d} \times 100 \)
- Predictive value of positive test = \( \frac{a}{a+b} \times 100 \)
- Predictive value of negative test = \( \frac{c}{c+d} \times 100 \)

Significance of the test has been calculated by calculating p-value.

**Observation and results:** Out of 70 cases, pleural fluid effusions were thirty one, peritoneal fluid effusions were thirty four and pericardial effusions were five. In a total of 70 patients, 43 males and 27 females were included in this study. Ages of the patients ranged from eight years to ninety years with maximum cases in the age group of 41 to 60 years (45.7%) and mean age of 50.36 years (Figures 6 and 7).

**Cytologic study:** Total leucocyte count: The total leukocyte count ranged from 57 to 1,50,000 cells/mm\(^3\). The estimated mean±SD of pleural, peritoneal, pericardial fluid cell count of all 70 cases were 3151.5 ± 17974.06. This was statically not significant (p value = 0.141). The average cell count in malignant effusion, chronic effusion and inflammatory effusion was 620 cells/mm\(^3\), 520 cells/mm\(^3\) and 31,141 cells/mm\(^3\) respectively (Figures 8-10). Lymphocytes were predominantly seen in patients with chronic effusion. Lymphocytes were also seen in cases of malignant effusion. Five patients with malignant effusion showed malignant cells in fluid cytology. The estimated mean ± standard deviation of all the transudative fluid total leukocyte count were 188.57 ± 53.65 and of exudative fluid cell count were 5793.29 ± 24576.28. This was statistically not significant (p-value = 0.141 using chi-square test).

**Radiology:** All the patients of pleural fluid effusion underwent chest x-ray PA view. Patients of peritoneal effusion were not underwent chest x-ray. Only some patients of pericardial effusion underwent chest x-ray PA view. Evidence of pleural effusion was seen in the chest x-ray in all cases of pleural fluid effusion.

Right lower lobe pneumonia with right sided effusion was present in four cases. Right sided pleural effusions were seen in thirteen cases. Left fibrocavity lesion with left sided effusion were seen in two cases. Left apical fibrosis with left sided pleural effusion was present in four cases. Left lobe in infiltration was present in one case. Cardiomegaly with right sided pleural effusion was present in three cases. Mass lesion in lung with right sided pleural effusion was present in one case. Massive pericardial effusion was present in one case.

Left sided pleural effusion was present in three cases. Chest x-ray PA view not done in 38 cases.

**Biochemical studies:**

1. **Appearance:** Out of 70 cases 50% were turbid, 47.1% were clear and 2.9% were hemorrhagic in appearance

2. **The following biochemical parameters of pleural fluid, peritoneal fluid and pericardial fluid were analyzed together in the present study:**
   a. Pleural fluid, peritoneal fluid and pericardial fluid glucose
   b. Pleural fluid, peritoneal fluid and pericardial fluid total protein.

3. **Transudative effusion and exudative effusion:** Based on the value of fluid total protein, the total cases were divided into transudative effusion and exudative effusion. If fluid total protein is less than 3 gm/dl (< 3 gm/dl), glucose less than 60
mg/dl ( < 60 mg/dl), then it is transudative fluid and if fluid total protein is more than 3 gm/dl, glucose > 60 mg/dl then it is exudative fluid.

In the present study out of 70 cases, 47.1% of effusion were transudative and 52.9% were exudative. Here, exudates exceed transudates. Most of the transudative effusion were clear and most of the exudative effusion were turbid in appearance.

The mean fluid glucose level on transudative effusion were in the range of 80.15 ± 21.19 mg/dl which was higher as compared to exudative effusion and this difference was statistically highly significant (p value = 0.010).

The mean fluid total protein levels in transudative effusion were in the range of 2.08 ± 0.43 gm/dl which can be classified as transudative form as compared to exudative form and this is highly statistically significant (p value = 0.001).

Peritoneal fluid with clinical and cytologic diagnosis: Out of 34 cases of peritoneal fluid, 32 cases are found cytologically as chronic effusions (94.1%) of which 24 cases are diagnosed clinically as cirrhosis of liver (70.6%) followed by two cases of malignant effusions (5.9%) of which one case was of carcinoma of ovary (2.9%) and one case was of carcinoma of gallbladder (2.9%) (Figure 11 and Table 10).

Chest x-ray was not done in all the 34 cases: Out of 34 cases, 24 cases are transudates (70.6%) and 10 cases are exudates (29.4%). 24 cases are clear (70.6%) in appearance followed by nine cases are of turbid (26.5%) in appearance and one case was hemorrhagic (2.9%) in appearance.

Pericardial fluid analysis

Out of 70 cases, only five cases are of pericardial fluid. Four patients are males (80%) and one patient was females (20%). Most of the patients are of 21 to 40 years age group. The most common presenting complain was chest pain, shortness of breath, loss of appetite and loss of weight (80%) followed by cough, fever, chest pain, shortness of breath, loss of appetite and loss of weight (20%). All the five cases were clinically diagnosed as pericardial tamponade of which three cases are diagnosed cytologically as chronic effusion (60%) and two cases as malignant effusion (40%). Predominant cell type on pericardial fluid total leukocyte count was lymphocyte in all the cases. Chest x-ray was done in only one case which was found as massive pericardial effusion (20%). Out of five cases, four cases are exudates (80%) and one case was transudate (20%). 80% of fluid are turbid and 20% are clear in appearance (Table 11). Cytologically, there are 59 cases of chronic effusions, six cases of inflammatory effusions and five cases of malignant effusions. Out of five cases of malignant effusion only two cases was found to be malignant mesothelioma. Among eight cases which were described as abdominal tuberculosis on clinical diagnosis, cytologically all eight cases were found to be chronic effusion. Out of 30 cases which were described as cirrhosis of liver on clinical diagnosis, cytologically all 30 cases were found to be chronic effusion. Among 15 cases which were described as pulmonary tuberculosis on clinical diagnosis, cytologically all 15 cases were found to be chronic effusion. Among three cases which were described as carcinoma of gall bladder, carcinoma of ovary and carcinoma of lung on clinical diagnosis, cytologically also all three cases were found to be malignant effusion. Among five cases which were described as pericardial tamponade on clinical diagnosis, cytologically two cases are found to be malignant effusion while three cases are chronic effusion. Out of those two cases of malignant effusion, one case was found to be malignant mesothelioma. Among two cases

Figure 8: Malignant cells in peritoneal fluid cytology (PAP, x200).

Figure 9: Malignant cells in peritoneal fluid cytology (PAP, x400).

Figure 10: Malignant cells in pericardial fluid cytology (PAP, x200).
which were described as empyema on clinical diagnosis, cytologically two cases were found to be inflammatory effusion. Out of four cases which were described as pneumonia on clinical diagnosis all four cases were found to be inflammatory effusion. Among three cases which were described as congestive cardiac failure on clinical diagnosis, cytologically all three cases were found to be chronic effusion. There was statistically significant correlation between clinical diagnosis and cytological diagnosis (p value < 0.05) (Figures 12-15 and Table 12).

From the above table, the following statistical parameters were calculated.

1. Sensitivity = TP / (TP+FN) = 60%
2. Specificity = TN/ (TN+FP) = 96.92%
3. Positive predictive value = TP/ (TP+FP) = 60%
4. Negative predictive value = TN/ (TN+FN) = 96.92%
5. Accuracy = TP+TN/ (TP+FP+TN+FN) = 94.28%

After comparison of results of cytology with clinical diagnosis
Selective centers, and its cost is beyond the reach of an average person, diseases, but it is invasive and expensive procedure, with a risk of safe and effective procedure for the diagnosis and therapy of pleural diagnoses [43]. Hence discrimination of pleural fluid as a transudate or exudates clearance of accumulating fluid, infection or bleeding in pleural space suffering from pulmonary or cardiac problems. Pleural effusion may be a transudate, if the cause is due to increased hydrostatic pressure pulmonary or cardiac disease. Hence our study is in concordance with the study of Alusi [5].

The relative ease of pleural fluid aspiration, analysis and cytological examination has kept alive the search for a test to unequivocally differentiate the various causes of effusion. The cytological study examination of body effusion is a complete diagnostic modality which aims at pointing out the etiology of effusions. The diagnostic performance of cytological study of the fluid may be attributable to the fact that the cell population present is representative of a much larger area than that obtained by needle biopsy [45].

Cytological examination of body fluids (pleura, pericardial and peritoneal fluid) have been universally recognized as the important diagnostic tool in the recognition of malignant tumors in effusions. In malignant disease, the cytological examination of fluid is by far more accurate than a pleural biopsy. In infectious diseases, particularly in tuberculosis, the pleural biopsy is superior to cytologic examination [46]. Immunocytochemistry is an essential adjunct to cytomorphology in selected cases and substantially improves diagnostic accuracy [47]. The cytological diagnostic of coelomic fluid is essential for malignant mesothelioma (MM). However, reactive mesothelioma (RM), caused by various factors, is morphologically similar to MM and RM and thus often complicates the differential diagnosis [48]. In a study conducted by Lovrenski et al. (2012) suggests that low sensitivity of cytological examination, recommend to perform biopsy of pleura for definitive diagnosis in every patient with clinical symptoms and suspicious radiography [49].

The present study showed slight male preponderance over female in pleural fluid study, male (58.1%) and female (41.9%). Similar comparative study by Romero et al. [50] with male preponderance in their study. Burgess et al. [51], in his study used biochemical parameters to distinguish between pleural exudates and transudates. In 393 cases, 270 were exudates and 123 were transudates. Valdes et al. [52] studied 253 pleural effusions, of which transudate (65 cases), neoplastic (37 cases), tuberculous (65 cases), and miscellaneous exudates (56 cases).

The most frequent cause of exudative effusion was pulmonary tuberculosis (45.2%) followed by pneumonia (12.9%), empyema (6.5%) and malignancy (3.2%). Remaining cases were due to liver diseases and heart disease. Hence our study is in concordance with the study of Light and Erozan [53].

Transudative effusions are usually characterized by majority of lymphocytes or other mononuclear cells. In our study also 80% of samples of transudative effusion had more than 50% lymphocytes. When exudative effusions are considered all tuberculous effusion had more than 50% small lymphocytes. Thus our study correlate with the study of Alusi [5].

The presence of predominantly polymorphonuclear cells in pleural fluid indicates that the fluid is the result of acute inflammation, hence raising the probability of pneumonia with effusion. In the present study we had not found eosinophils in the pleural fluid. Undoubtedly, there are other factors involved since a significant number of eosinophilic effusion are non-hemorrhagic and not all hemorrhagic effusions are eosinophilic [54].

The study included 200 patients with pleural effusion. Pleural effusions were transudative in 48 (24%) and exudative in 152 (76%) of cases. Congestive cardiac failure (14.5%), nephrotic syndrome (5.5%),

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Chronic effusion</th>
<th>Inflammatory effusion</th>
<th>Malignant effusion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis of liver</td>
<td>24 (70.6%)</td>
<td>8</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Abdominal tuberculosis</td>
<td>8 (23.6%)</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma of gallbladder</td>
<td>1 (2.9%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma of ovary</td>
<td>1 (2.9%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32 (94.2%)</td>
<td>2 (5.8%)</td>
<td>34 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 10: Showing correlation of clinical and cytological diagnosis in peritoneal fluid.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Chronic effusion</th>
<th>Inflammatory effusion</th>
<th>Malignant effusion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Tuberculosis</td>
<td>8 (11.4%)</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis of Liver</td>
<td>15 (21.4%)</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empyema</td>
<td>2 (2.9%)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4 (5.7%)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma of gall bladder</td>
<td>1 (1.4%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCF</td>
<td>3 (4.3%)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial tamponade</td>
<td>3 (4.3%)</td>
<td>2 (2.8%)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Carcinoma of lung</td>
<td>1 (1.4%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma of Ovary</td>
<td>1 (1.4%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>6</td>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 11: Showing correlation of clinical diagnosis and cytological diagnosis.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Neoplastic</th>
<th>Non-neoplastic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3 (True Positive)</td>
<td>2 (False positive)</td>
<td>5</td>
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<tr>
<td>Negative</td>
<td>2 (False Negative)</td>
<td>63 (True Negative)</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>65</td>
<td>70</td>
</tr>
</tbody>
</table>

P-Value = 0.003

Table 12: Overall performance of cytology in diagnosis of pleural, peritoneal and pericardial effusion.

Overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value (PPV) was 60% and negative predictive value was 96.92%. The study was statistically significant (p value = 0.003).

Discussion

Pleural effusion is a frequently encountered problem in patients suffering from pulmonary or cardiac problems. Pleural effusion may be a transudate, if the cause is due to increased hydrostatic pressure or decreased osmotic pressure in the pleural cavity; may be exudates, if there is abnormal pleural capillary permeability, reduced lymphatic clearance of accumulating fluid, infection or bleeding in pleural space [43]. Hence discrimination of pleural fluid as a transudate or exudates remains the basic diagnostic algorithm for exudates, malignancy, bacterial pleurisy and tuberculous effusions are the principal differential diagnoses [43].

Medical thoracoscopy, in trained hands of a pneumologist is a safe and effective procedure for the diagnosis and therapy of pleural diseases, but it is invasive and expensive procedure, with a risk of complications like pneumothorax. Moreover, it is available at very selective centers, and its cost is beyond the reach of an average person, in developing countries like Nepal. Moreover, a recent survey revealed that even in USA, only 6% of pneumologists are currently trained in and perform this valuable procedure [44]. By contrast, thoracoscopy has been a very popular diagnostic as well as therapeutic procedure for tapping pleural effusions and has very less procedural complications.

Immunocytochemistry is an essential adjunct to cytomorphology, in selected cases and substantially improves diagnostic accuracy [47]. The cytological diagnostic of pleural effusion is essential for malignant mesothelioma (MM). However, reactive mesothelioma (RM), caused by various factors, is morphologically similar to MM and RM and thus often complicates the differential diagnosis [48]. In a study conducted by Lovrenski et al. (2012) suggests that low sensitivity of cytological examination, recommend to perform biopsy of pleura for definitive diagnosis in every patient with clinical symptoms and suspicious radiography [49].

The present study showed slight male preponderance over female in pleural fluid study, male (58.1%) and female (41.9%). Similar comparative study by Romero et al. [50] with male preponderance in their study. Burgess et al. [51], in his study used biochemical parameters to distinguish between pleural exudates and transudates. In 393 cases, 270 were exudates and 123 were transudates. Valdes et al. [52] studied 253 pleural effusions, of which transudate (65 cases), neoplastic (37 cases), tuberculous (65 cases), and miscellaneous exudates (56 cases).

The most frequent cause of exudative effusion was pulmonary tuberculosis (45.2%) followed by pneumonia (12.9%), empyema (6.5%) and malignancy (3.2%). Remaining cases were due to liver diseases and heart disease. Hence our study is in concordance with the study of Light and Erozan [53].

Transudative effusions are usually characterized by majority of lymphocytes or other mononuclear cells. In our study also 80% of samples of transudative effusion had more than 50% lymphocytes. When exudative effusions are considered all tuberculous effusion had more than 50% small lymphocytes. Thus our study correlate with the study of Light and Erozan [53].

The presence of predominantly polymorphonuclear cells in pleural fluid indicates that the fluid is the result of acute inflammation, hence raising the probability of pneumonia with effusion. In the present study we had not found eosinophils in the pleural fluid. Undoubtedly, there are other factors involved since a significant number of eosinophilic effusion are non-hemorrhagic and not all hemorrhagic effusions are eosinophilic [54].

The study included 200 patients with pleural effusion. Pleural effusions were transudative in 48 (24%) and exudative in 152 (76%) of cases. Congestive cardiac failure (14.5%), nephrotic syndrome (5.5%),


ISSN: 2157-7099 JCH, an open access journal
and liver cirrhosis (2.5%) were the most common etiological diagnoses of transudate cases. Malignant effusion (16.5%), pneumonia (13%), pleural empyema (9%), tuberculosis (6%), and pulmonary embolism (5.5%) were the most common etiological diagnoses of exudative cases.

Thirty-two (16%) cases of exudative pleural effusions were of undetermined etiology. Polymorphonuclear leukocytes predominated in 48 patients with exudative pleural effusions. The most common etiological diagnoses were pneumonia (41.67%), pleural empyema (39.59%) and pulmonary embolism (10.42%). Lymphocytes in pleural fluid were predominant in 63 patients, with malignant (6.34%), tuberculous pleurisy (19.02%), pulmonary embolism (6.34%), trauma (6.34%), and (46.11%) cases in patients with pleural exudate undetermined etiology. Eosinophils were predominant in 16 (8%) patients with exudative pleural effusions. The most common etiology of eosinophilic pleural fluid were pneumonia (37.5%), malignant pleural effusion (25%), pulmonary embolism (12.5%), pyopneumothorax (6.25%) and trauma 6.25%. From 16 patients with eosinophilic pleural effude, in 31% cases air, in 12.5% blood in pleural fluid were determined and in 12.5% cases previous pleural puncture was performed. Pleural fluid eosinophilia is most commonly associated with the presence of air or blood in the pleural fluid (correlation index 0.82). Malignant pleural effusions were determined in 33 patients. Malignant cells in pleural fluid were identified in 25 cases. The diagnostic sensitivity of pleural fluid cytology for malignant pleural effusions were 76%. Hemoragic pleuritis was determined in 18 and hemotorax in 4 patients. Etiology of hemotorax were trauma (75%) and coagulopathia (25%). Most common etiological diagnoses of hemoragic pleuritis were neoplasia (33.3%), pulmonary embolism (16.65%), trauma (16.65%), pneumonia (11.11%), and congestive cardiac failure (11.11%). Diagnostic sensitivity and specificity of hemoragic pleuritis is low, 58% and 45% respectively [55]. Many types of cancer metastasize to the epithelial linings of the body cavity causing malignant fluid to accumulate in such spaces. Cytomorphological examination is considered essential in the diagnosis of malignant body fluid. Nevertheless, the accuracy of cytomorphological results is subjective and can vary depending on the cytopathologists’ experience [56]. The study conducted by Swamy et al. [57], 200 patients of pleural fluid effusion were included in the study. Of which 125 males and 75 females, age ranged from ten years to seventy years, with maximum cases (30%) in the sixth decade. Of all the effusion 30.5% were hemorrhagic, 28.5% turbid and 24% clear. Transudates comprised 20% of cases. Most of them were secondary to cirrhosis of liver and congestive cardiac failure. Overall 80% cases of pleural effusion were exudative in nature. In our study out of 70 cases of pleural, peritoneal and pericardial fluid, 31 cases were of pleural effusion, 18 were males (58.1%) and 13 were females (41.9%). Of all the effusion 74.2% are exudates and 25.8% are transudates. Of all effusion 71.0% were turbid followed by clear (25.8%) and hemorrhagic (3.2%).

Hence our study was concordance with the study of Swamy et al. In a study conducted by Prabhudesai et al. [58] consecutive patients over the age of 40 years with exudative pleural effusion that malignant effusion was most common found in 49 patients (64.47%) and infective cause accounted for 24 of the total effusion (31.57%).

There are significant differences between the diagnostic value of various pleural fluid and serum markers. Overall, pleural fluid markers are superior to serum markers in determining the pleural fluid etiology. A combination of two or more tumor markers may help improve their diagnostic accuracy. Pleural fluid and serum measurements of different tumor markers play a limited role in the differentiation between malignant and non-malignant pleural effusions [59].

In a study conducted by Gornik et al. [60], 219 patients underwent pericardiocentesis of which 43.8% of cases of effusion was found cancer-related. In our study out of five cases of pericardial fluid only 40% of cases (two cases) are cytologically diagnosed as malignant effusion. Both malignant effusion are of malignant mesothelioma. Hence present study was concordance with others study.

In a study conducted by Mitsu et al. [11] fiftythree specimens of pericardial fluids were obtained from 21 males and 23 females patients. The age range was from 16 to 91 years (average, 56.9 years). Cytomorphological examination resulted in the following diagnoses: negative for malignancy, 28 cases; suspicious for malignancy, three cases and 13 cases, positive for malignancy [11]. In our study five specimens of pericardial fluid were obtained from four males and one female, the age range from 21 to 40 years. Of the five cases, two cases are cytomorphologically resulted as positive for malignancy (malignant mesothelioma) followed by three cases, negative for malignancy.

In a study conducted by Sears et al. [61] 3011 pleural and peritoneal effusion specimens were examined over a three-year period (1982 to 1984). Totals of 812 (44%) of 1,846 pleural and 423 (36%) of 1,165 peritoneal specimens were positive for malignant cells. In our study out of 70 cases of pleural, peritoneal and pericardial fluid specimens were examined.61 Out of 31 cases of pleural fluid only one case (3.2%) was positive for malignant cells and out of 34 cases of peritoneal fluid only two cases (40%) were positive for malignant cells. Hence our study was concordance with their study.

In a study conducted by Triol et al. [62], 75 cases of diffuse pleural and/or peritoneal malignant mesothelioma were studied by cytologic methods, of which 64% of the cases were cytologically diagnosable mesothelioma and 42% of cases in which the cytomorphic impression was uncertain or equivocal [62]. In our study of the total 34 cases of peritoneal fluid studied cytologically only two cases (5.8%) resulted as positive for malignancy (malignant effusion) which were clinically diagnosed as carcinoma of ovary and carcinoma of gallbladder.

In a study conducted by Khan et al. [63,54] patients with peritoneal (abdominal) tuberculosis was identified, the mean age of them was 31.85 years and 96.3% (52/54) of them with male predominance. The main symptoms at the time of presentation were abdominal pain and abdominal distension (ascites). In the present study out of 34 cases of peritoneal fluid, eight (23.6%) cases were of peritoneal (abdominal) tuberculosis. The mean age of the patients was 52.50 years and 61.8% with male preponderance. The main symptoms at the time of presentation were abdominal distension (ascites), swelling of lower limbs, yellowish discoloration of sclera and loss of weight. Hence our study concordance with others study.

The present study was concordance with the study of Yoneshima et al. [64] with eight cases of peritoneal tuberculosis. Three patients were males and five were females, age ranged between 28 and 80 years old, abdominal distension seen in four patients and loss of appetite in five patients.

In a study conducted by Wu et al. [65], 17 patients diagnosed as peritoneal tuberculosis were taken, ageranged from 24 to 87 years (median, 38 years),only seven patients underwent paracentesis and ascitic fluid were cytologically analysed. In that cases cytology revealed lymphocyte-dominant ascites without malignant cells. In the present study eight cases were diagnosed as peritoneal (abdominal) tuberculosis with cytologic findings lymphocyte predominant ascites without malignant cells.
In a study conducted by Kundu, et al. [66], 100 effusions (55 pleural, 44 peritoneal and one pericardial fluid) were studied by cytology, Epithelial membrane antigen (EMA) and Flowcytometry (FCM). Out of hundred cases, 29 cases were malignant and 71 benign cases. On cytology 28 of 29 malignant cases were diagnosed with no false positive, the sensitivity and specificity was 96.55% and 100% respectively. The p value was less than 0.05 (< 0.05) [66]. In the present study seventy effusions (31 pleural, 34 peritoneal and five pericardial fluid) were studied only cytologically. On cytology five cases were diagnosed as malignant effusion and sixty five benign cases, with two false positives, the sensitivity and specificity was 60% and 96.92% respectively. This was statistically significant (p value < 0.003). In the present study EMA and FCM analysis was not done.

Cytologic specimens of 105 pericardial fluids collected from 95 cases during a seven-year period were reviewed. Clinical reports and descriptions of the histologic antemortem and postmortem specimens were correlated with the cytologic diagnoses, and the interobserver variation was estimated. Of the collected material, 48.4% was from patients suspected of having nonmalignant disorders, 40.0% was from patients with previously diagnosed carcinoma and 11.6% was from cases in which the etiology was unknown at the time of pericardiocentesis. Cytologic examination of the pericardial fluid revealed tumor cells in a sample from one patient suspected of having a heart disorder and in a sample from another patient with an obscure disease. Of the pericardial fluids from the cancer patients, 66.7% contained malignant cells; the most frequent primary site in these cases was the lung. Correlated with the histologic diagnosis, the specificity of cytology was 100%. The results prove that, in experienced hands, pericardial cytology is a valuable diagnostic tool [67].

In a study conducted by Hwangbo et al. [68], 366 patients were underwent diagnostic paracentesis and etiology was confirmed by histology, imaging studies and peritoneal fluid analysis. They found that 59.6% were cirrhotic ascites, 25.7% cancer related, 6.6% tuberculous peritonitis and others 8%. In the present study out of 34 cases, 24 (70.6%) cases were cirrhotic ascites, eight (23.6%) cases abdominal tuberculosis (peritoneal tuberculosis) and two (5.8%) cases cancer related.

In a study conducted by Romney et al. [69,67] cirrhotic patients had underwent therapeutic paracentesis, an ascitic fluid analysis revealed only neutrophils as a predominant cell type. In the present study cirrhotic ascites show predominantly lymphocytes but neutrophils were not present in the fluid. In a patient with acute abdominal pain, abdominal paracentesis and diagnostic peritoneal lavage often yield fluid samples for cytologic and biochemical evaluation. Cytology of the effusion from a patient with acute abdominal diseases can be crucial tool for the rapid diagnosis necessary for initiation of timely and appropriate therapy [70].

By monitoring the ascitic fluid polymorhonuclear cell (PMN) count it seems to be possible to determine the efficacy of some drug (cefotaxime) therapy in patients with spontaneous bacterial peritonitis [71].

In a study conducted by Dragoescu et al. [72], total 128 pericardial fluid specimens were obtained from 113 patients (56 males and 57 females), representing 4.5% of all fluids. Of these, 95 cases (74.2%) were benign, two cases (1.6%) had severe atypical cells and 31 cases (24.2%) were malignant [72]. In the present study only five cases of pericardial fluid specimens were obtained. Out of five cases (four males and one female, age ranged from 21 to 40 years) cytologically three cases (60%) are diagnosed as chronic effusion and two cases (40%) were found as malignant effusion. Both are malignant mesothelioma. In the present study patients were not on follow-up and pericardial biopsy was not obtained from the patient having malignant effusion.

In a study conducted by Pawlak et al. [73], out of 191 patients (100 men and 91 women) age ranged from 19 to 88 years, only 93 cases underwent pericardiocentesis and pericardial fluid was examined. Out of 93 cases only 52 cases were cytologically diagnosed as malignant effusion. In the present study out of five cases only two cases are of malignant effusion. Pericardial effusion with cardiac tamponade can present as a primary pericardial mesothelioma [74]. Malignant mesothelioma is an asbestos-related malignancy that arises primarily from mesothelial cells on the serosal surfaces of the pleura, peritoneal and pericardial cavities. Morphological discrimination between malignant pleural mesothelioma and reactive mesothelial hyperplasia is difficult. Cytological analysis of pleura effusion is valuable for early diagnosis [75].

In a study conducted by Urrunaga et al. [76], 1113 cirrhotic patients with ascites underwent paracentesis and only 214 cases (19%) had hemorrhagic ascites. Patients with hemorrhagic ascites is a marker of advanced liver disease and poor outcome. In the present study twenty four cases of cirrhotic ascites underwent paracentesis and only one case (2.9%) had hemorrhagic ascites. In present study after comparison of results of cytology with clinical diagnosis overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value (PPV) was 60% and negative predictive value was 96.92%. Hence the study was statistically significant (p-value = 0.003).

**Statistical Analysis**

After comparison of results of clinical diagnosis with cytological diagnosis, overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value was 60% and negative predictive value was 96.92 %. Hence the study was statistically significant (p value = 0.003).

In a study conducted by Urrunaga et al., 69 overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value (PPV) was 60% and negative predictive value was 96.92%. In a study conducted by Kundu et al. [60] the sensitivity and specificity of cytology was 96.55% and 100% respectively. The p-value was less than 0.05 with no false positive.

**Summary and Conclusion**

The present study had a series of 70 cases of body fluid specimens including pleural fluid, pericardial fluid and peritoneal fluid.

- Age of the patients ranged from eight years to ninety years with mean age of 50.36 years with males to female ratio of 1:6:1.
- Majority of patients were presented clinically as abdominal distension, yellowish discoloration of sclera, swelling of lower limbs, shortness of breath, chest pain, loss of appetite and loss of weight. The most common presenting complain were abdominal distension, swelling of lower limbs and yellowish discoloration of sclera (42.9%) followed by cough, fever, chest pain, shortness of breath, loss of appetite and loss of weight. A common complaint was abdominal distension, swelling of lower limbs and yellowish discoloration of sclera (42.9%) followed by cough, fever, chest pain, shortness of breath, loss of appetite and loss of weight (12.9%) followed by chest pain, shortness of breath, loss of appetite and loss of weight (7.1%) followed by cough,
fever, and chest pain (5.7%) followed by loss of appetite and loss of weight (1.4%).

- Out of 70 cases, 34 cases are of peritoneal fluids, 31 cases of pleural fluids and five cases of pericardial fluids.
- Out of 70 cases, 59 cases diagnosed as chronic effusion (84.28%). Six cases are diagnosed as inflammatory effusion (8.58%) and five cases are of malignant effusions (7.14%) on cytological evaluation. Out of five cases of malignant effusion two cases are of malignant mesothelioma.
- Of the total 70 cases of effusion, 50% were turbid, 47% were clear and 2.9% were hemorrhagic in appearance. 52.9% of effusions were exudative and 47.1% of effusion were transudative. The mean fluid glucose level on transudative effusion were in the range of 80.15 ± 21.19 mg/dl which was higher as compared to exudative effusion and this difference was statistically highly significant (p value = 0.010). The mean fluid total protein levels in transudative effusion were in the range of 2.08 ± 0.43 gm/dl which can be classified as transudative form as compared to exudative form and this is highly statistically significant (p value = 0.001).
- The total leukocyte count ranged from 57 to 1,50,000 cells/mm3. The estimated mean ± SD of pleural, peritoneal, pericardial fluid cell count of all 70 cases were 3151.5 ± 17974.06. This was statistically not significant (p value = 0.141). The average cell count in malignant effusion, chronic effusion and inflammatory effusion were 620 cells/mm³, 520 cells/mm³ and 31,141 cells/mm³ respectively.
- Lymphocytes were predominantly seen in patients with chronic effusion.
- Lymphocytes were also seen in cases of malignant effusion. Five patients with malignant effusion showed malignant cells in fluid cytology. The estimated mean ± standard deviation of all the transudative fluid total leukocyte count were 188.57 ± 53.65 and of exudative fluid cell count were 5793.29 ± 24576.28. This was statistically not significant (p value = 0.141 using chi-square test).
- After comparison of results of cytology with clinical diagnosis overall sensitivity of cytology was found to be 60%, specificity as 96.92% and accuracy as 94.28%, while positive predictive value (PPV) was 60% and negative predictive value was 96.92%. There was statistically significant correlation between cytologic results and clinical diagnosis (p value < 0.003).

Currently biochemical parameters are used for classifying pleural, pericardial and peritoneal effusion along with newer cyto-chemical staining techniques. The diagnostic yield may increase upto 80% by these methods. Hence attempts have been made to identify markers, which allow a more accurate and rapid diagnosis. Though detailed clinical history and clinical signs give a clue to etiology of pleural, pericardial and peritoneal effusion, radiological, biochemical and cytological evaluation helps in narrowing down the diagnosis and management of the patient.

References
