INTRODUCTION

Pleural effusion (PE) is the most common manifestation of pleural involvement in many diseases. Pleural effusion formed due to malignancy is termed malignant pleural effusion (MPE) and the most common causes of MPEs are primary lung and breast cancer ***(Roberts et al., 2010).***

In the context of development of a pleural effusion, many factors pertinent to cancer that alter the permeability of the pleura and its microvasculature come into play, ranging from the protein content in the pleural fluid to stress hormones and growth factors ***(Zarogiannis et al., 2013).***

Sestrin-2 belongs to a family of highly conserved antioxidant proteins transcriptionally regulated by tumor suppressor p53, the most commonly mutated gene in cancers ***(Budanov, 2011).***

Overexpression of Sestrin-2 can suppress the target rapamycin (TOR) complex-1 (TORC-1) pathway that is often activated in human cancers, while in lung adenocarcinomas Sesn2 expression is down-regulated ***(Garber et al., 2001).***

Moreover, Sestrin2 may have both tumor-suppressive and metastasis promoting activities when counteracting with transforming growth factor beta (TGF-β) ***(Wempe et al., 2010).***

In pleural effusions, it has been shown that components of the oxidant–antioxidant equilibrium are capable of altering pleural permeability as well as to be overexpressed in exudative pleural effusions rendering support to the notion that oxidative stress may be a critical component of the pleural effusion formation ***(Papageorgiou et al., 2005).***

AIM OF THE WORK

To assess the level of Sestrin2 in patients with pleural effusions in order to differentiate between malignant pleural effusions (MPEs) and benign pleural effusions (BPEs).

PLEURAL EFFUSION

The visceral pleura envelopes the entire surface of both lungs except at the hilum, where the bronchi, pulmonary vessels and nerves enter the lung substance. The parietal pleura covers surface of the chest wall, mediastinum and diaphragm ***(Von Hayek, 1969)***.

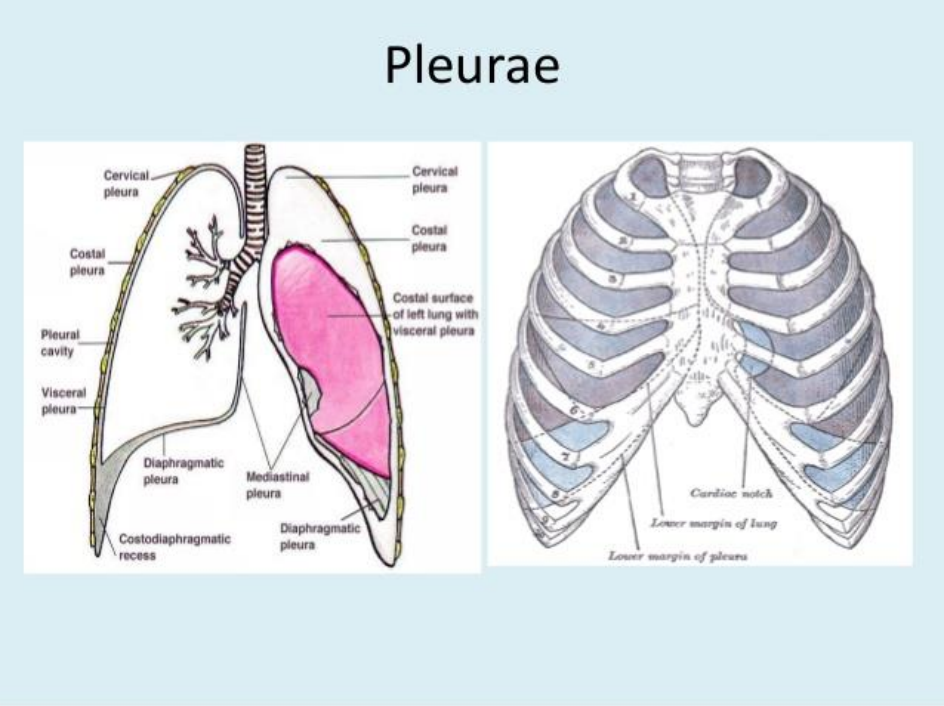

Figure (1): Anatomy of pleura ***(Von Hayek, 1969)***.

Below the merger of visceral and parietal pleura at the hilum, pleural reflections from the dorsal and ventral surface of the lungs usually extend to the diaphragm as a double layer of mesothelial tissue, the pulmonary ligament ***(Yunus et al., 2015).***

The normal pleural linings are smooth, glistening, semitransparent membranes. Beneath the one single layer of mesothelium is a band of connective tissue which contains abundant collagen and elastin. Mesothelial cells vary in size and shape, from flat to columnar. Many mitochondria, rough endoplasmic reticulum and golgi apparatus are eminent features of both cuboidal and columnar cells indicating that they are active in transporting substances across the pleural surfaces and in ensuring the structure and function of the pleural space ***(Yunus et al., 2015).***

Microvilli project from the mesothelial cells serve as a lubricant and to increase the surface area of the pleura for fluid transport ***(Yunus et al., 2015).***

Important anatomic differences are found beneath the similar external appearance of both the parietal and visceral pleura. Beneath the parietal surface, the arrangement of the connective tissue layer is straight forward; while, the submesothelial connective tissue layer of the visceral pleura gives rise to many septae that permeate the lungs creating subdivisions that promote gas exchange while supporting the pulmonary parenchyma ***(Finley and Rusch, 2011).***

The visceral pleura is not innervated,where as the parietal pleura is innervated by nerve fibres from different origins. The costal pleura and the periphery of diaphragmatic pleura are innervated by the intercostal nerves; painful stimuli

in these regions elicit pain in the adjacent chest wall. The central parts of the diaphragm are innervated by the phrenic nerve; stimuli in these areas are sensed in the ispsilateral shoulder ***(Finley and Rusch, 2011).***

The blood supply is different in both the visceral and the parietal pleura. Systemic arteries supply the parietal pleura depending on their location. Branches from these arteries form a network beneath the mesothelium of costal and mediastinal pleura ***(Finley and Rusch, 2011).***

While, there is a dual origin of blood supply to the visceral pleura from bronchial and pulmonary circulations. Whatever the origin of blood supply, the capillaries drain into the pulmonary veins ***(Finley and Rusch, 2011).***

The lymphatic drainage of visceral and parietal pleura is different. The parietal lymphatics are the main route of draining the pleural space. Its mesothelial surface is permeated by stomas that allow connection to the lymphatic network in the nearby submesothelial layer. The visceral pleura is devoid of stomas and the underlying lymphatic vessels appear to drain the pulmonary parenchyma and not the pleural space ***(Finley and Rusch, 2011).***

Physiology of the pleura:

Formation of Pleural Fluid:

Normally, the composition of pleural fluid depends on ultrafiltration of the plasma (see table 1). The two pleurae act as semipermeable membranes, so the concentrations of small molecules like glucose are similar in both pleural fluid and plasma. Whereas large molecules like albumin are lower in pleural fluid ***(Yunus et al., 2015).***

Table (1): Composition of pleural fluid ***(Yunus et al., 2015)***

Normal composition of pleural fluid	
Volume	0.1-0.2ml/kg
Cells/mm3	1000-5000
% mesthothelial cells	3-70%
% monocytes	30-75%
% lymphocytes	2-30%
% Granulocytes	10%
Protein	1-2g/dl
% albumin	50-70%
Glucose	Same as Plasma level
LDH	<50% of plasma level
PH	>plasma

Although the volume of fluid normally present in the pleural space is small (5 – 15 ml), the rate of turnover of pleural fluid is rapid and may exceed onelitre per day. Because the rates of fluid entry and exit are nearly equal, the amount of pleural fluid remains constant. This equilibrium is dependent on the forces employed in starling's equation for "transcapillary" exchange. According to this equation, net filtration or reabsorption of water (and its solutes) across a semipermeable membrane is determined by balances between the hydrostatic and oncotic pressures on both sides of the pleural surfaces, which are covered by porous mesothelium, the principal barrier to pleural fluid filtration and reabsorption is the endothelium of the pleural capillaries ***(Yunus et al., 2015).***

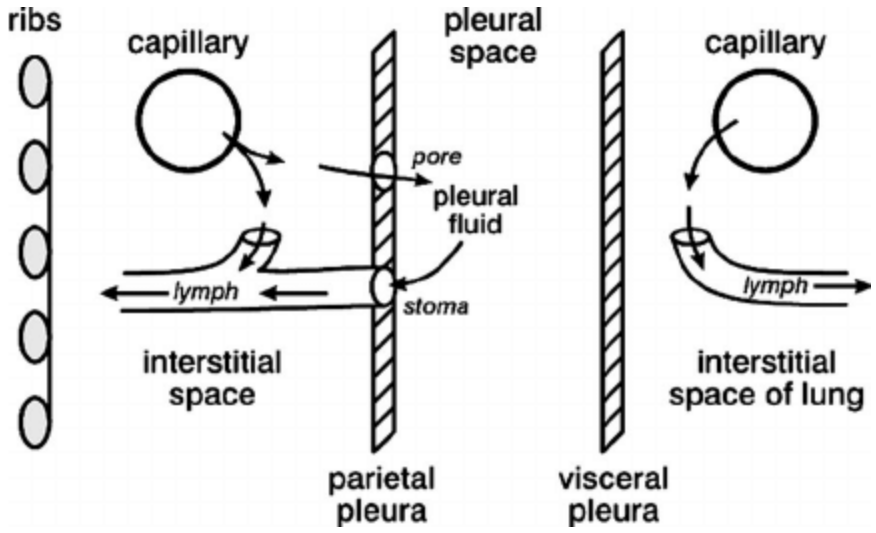

Figure (2): Diagrammatic representation of pleural fluid formation and the parietal and visceral pleura ***(Finley and Rusch, 2011).***

Disequilibrium in starling forces of the pulmonary lymphatics will lead to excess water and protein in pleural

fluid. For the pleura, starling's equation can be written as follows: ***(Yunus et al., 2015)***

$$F = K \{(P_{cap} - P_{pl}) - \sigma (\pi_{cop} - \pi_{pl})\}$$

Where:

F = rate of fluid movement

P and π = hydrostatic and oncotic pressures respectively

K = the filtration coefficient

σ = the osmotic reflection coefficient for protein, similar to that of muscle capillaries (equals about 0.9).

cap = Capillary

Pl = Pleural space.

The hydrostatic pressure within the capillaries of the parietal pleura is similar to that in other systemic capillaries (i.e., mean pressure about 25 mm Hg); intrapleural pressure is subatmospheric, about – 3mmHg. The resulting difference favours fluid filtration. Opposing this is the oncotic pressure difference due to the higher concentration of protein in plasma than in pleural fluid ***(Finley and Rusch, 2011).***

The osmotic force promoting the reabsorption of pleural fluid can be calculated as the product of the osmotic reflection coefficient for protein and the difference between the oncotic pressure of plasma and of pleural fluid:0.9 x (28 – 5) = 21 mmHg. Because of the hydrostatic pressure gradient in parietal

pleural capillaries exceeds the oncotic pressure gradient, fluid filters continuously into the pleural cavity ***(Finley and Rusch, 2011).***

In the visceral capillaries, the balance between hydrostatic and oncotic pressures is opposite even though oncotic pressures are the same as in parietal capillaries, the hydrostatic pressures in the visceral pleural capillaries are lower and are closer to those in the pulmonary capillaries (about 10 mm Hg). Therefore, the balance of hydrostatic and oncotic pressures promotes the reabsorption of fluid across the visceral pleural surfaces ***(Finley and Rusch, 2011).***

The mesothelium also plays a role in the reabsorption of fluid. Both sodium and chloride are actively transported out of the pleural fluid via a Na^+/H^+ and Cl^-/HCO_3^- double exchange on the serosal surface and a Na^+/K^+ pump on the interstitial side of the mesothelium ***(Finley and Rusch, 2011).***

The lymphatics in the parietal pleura protect against accumulation of excess fluid and recover proteins from pleural space to the plasma. The protein concentration in the capillaries of the parietal and visceral pleura exceeds that of pleural fluid, so a small quantity of proteins continually diffuses into the pleural fluid ***(Finley and Rusch, 2011).***

If there were no mechanism for removing protein from the pleural space, the oncotic gradient opposing the filtration of

fluid would dissipate and fluid would accumulate. The pleural lymphatics remove sufficient protein from the pleural cavity to maintain the difference between plasma and pleural fluid protein concentrations and thereby the volume of pleural fluid, relatively constant. Excess fluid in the pleural space increases lymph flow considerably. Conversely, lymphatic obstruction causes pleural fluid to accumulate ***(Finley and Rusch, 2011).***

Pleural effusion

Pleural effusion is an excessive accumulation of fluid in the pleural space, and indicates an imbalance between pleural fluid formation and removal. Accumulation of pleural fluid is not a specific disease, but rather a reflection of underlying pathology. Pleural effusions accompany a wide variety of disorders of the lung, pleura, and systemic disorders. Therefore, a patient with pleural effusion may present not only to a pulmonologist but to a general internist, rheumatologist, gastroenterologist, nephrologist, or surgeon. To treat pleural effusion appropriately, it is important to determine its cause ***(Karkhanis et al., 2012).***

With knowledge of the pleural fluid cytology, biochemistry, and clinical presentation, an etiological diagnosis can be established in approximately 75% of patients ***(Karkhanis et al., 2012).***

Common causes of pleural effusion are shown in the figure below in up to 20% of cases, the cause remains unknown despite a diagnostic workup ***(Light, 2006).***

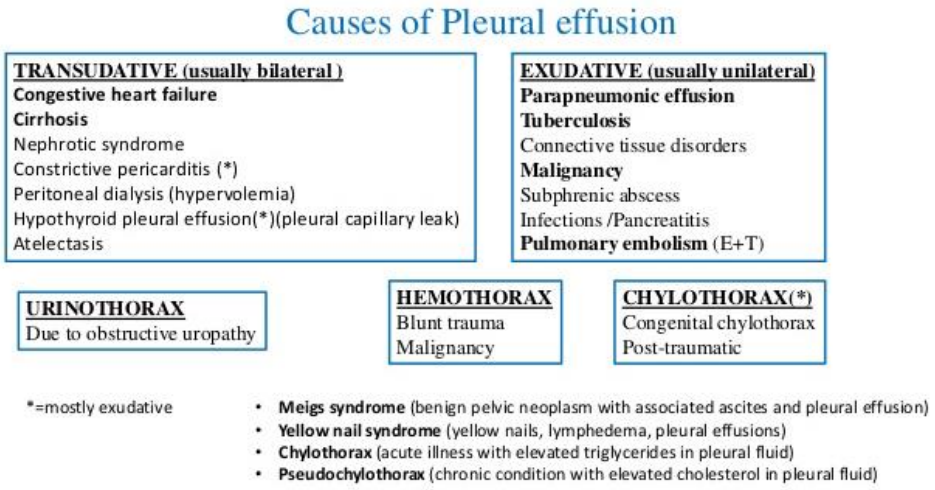

Figure (3): Causes of pleural effusion ***(Karkhanis et al., 2012).***

The clinical presentation of pleural effusion depends on the amount of fluid present and the underlying cause. Many patients have no symptoms at the time a pleural effusion is discovered. Possible symptoms include pleuritic chest pain, dyspnea, and a dry, nonproductive cough. The chest pain associated with pleural effusion is caused by pleural inflammation of the parietal pleura resulting from movement-related friction between the two pleural surfaces ***(Cassina et al., 1999).***

Pleuritic chest pain may be localized or referred. The pain is usually sharp and is exacerbated by movement of the pleural surfaces, as with deep inspiration, coughing, and sneezing. The pain eases with strapping of the chest or on

accumulation of fluid. Because dyspnea and chest pain are nonspecific symptoms, a careful history and physical examination are important in narrowing the differential diagnosis ***(Karkhanis et al., 2012).***

Diagnostic pleural tapping with biochemical, cytological, and microbiological examination of the fluid is needed for correct diagnosis. Differentiation between transudate and exudate is crucial before further tests are undertaken ***(Rodney et al., 1995).***

Characterization of pleural fluid as an exudate or transudate is an important step in pleural fluid analysis. Light's criteria are the most sensitive for identifying exudates, with 98% sensitivity and can differentiate between transudate and exudates ***(Karkhanis et al., 2012).***

Pleural fluid	PF/serum protein ratio	PF/serum LD ratio	PF LD (U/L)
Transudative	< 0.5	< 0.6	< 2/3 URL
Exudative*	≥ 0.5	≥ 0.6	≥ 2/3 URL

*Effusions are identified as exudative if one or more conditions are met.
LD – lactate dehydrogenase; PF – pleural fluid; URL – upper reference limit of serum LD.

Figure (4): Lights criteria for diagnosis of pleural effusion ***(Rodney et al., 1995)***

On the basis of Light's criteria, some patients who actually have a transudative pleural effusion will be thought to have an exudative pleural effusion. If the clinical appearance

suggests a transudative effusion, but the pleural fluid is an exudate according to Light's criteria, the difference between albumin levels in serum and in pleural fluid should be measured. Almost all patients with a serum albumin level 1.2 g/dL higher than the pleural fluid albumin level have a transudative effusion. These effusions are known as trans-exudative effusions ***(Karkhanis et al., 2012).***

Transudative effusion

A) Pleural effusion associated with congestive heart failure

Patients with congestive heart failure and pleural effusion present with orthopnea, paroxysmal nocturnal dyspnea, and on examination have fine crackles. Chest X-ray shows cardiomegaly and bilateral pleural effusions, generally the right effusion being larger than the left. These are transudative effusions but may present with transexudates in patients who are on diuretic therapy. The pleural to serum-effusion albumin gradient will be greater than 1.2 g/dL even after diuresis. Serum and pleural fluid NT-proBNP levels are significantly elevated in patients with pleural effusion owing to heart failure. Hence they are of high diagnostic value in the diagnosis of heart failure-related pleural effusion ***(Light, 2006).***

B) Pleural effusion associated with liver diseases

Hepatic hydrothorax is a pleural effusion that develops in a patient with chronic liver disease. Effusion is caused by passage of ascitic fluid from the peritoneal cavity into the

pleural space through diaphragmatic defects. Up to 20% of patients with hepatic hydrothorax have no clinically demonstrable ascites. Clinical signs of liver cirrhosis may be present. Effusions may be unilateral (17%) or bilateral (3%). Massive effusions occur in about 6% of patients. It is usually a serous or hemorrhagic transudate, with predominantly lymphocytes and mesothelial cells. Pleural fluid and ascitic fluid show similar biochemistry. Increasing effusion is often associated with a decrease in ascitic fluid ***(Karkhanis et al., 2012).***

C) Pleural effusion associated with nephrotic syndrome and renal failure

Approximately 20% of patients with nephrotic syndrome develop pleural effusion. Effusions result from severe hypoalbuminemia, which leads to decreased oncotic pressure. They are bilateral effusions, serous in nature with proteins <3 g/dL, with normal glucose and pH 7.4 ***(Karkhanis et al., 2012).***

Exudative pleural effusion:

A) Tuberculous pleural effusion

It is important to consider the possibility of tuberculous pleuritis in all patients with an undiagnosed pleural effusion. Tuberculous pleuritis is thought to represent primarily a hypersensitivity reaction to tuberculous protein, and the bacillary burden in the pleural space is low. Patients usually present with an acute illness. The most frequent symptoms are cough, which is nonproductive and associated with chest pain, which is