

## A COMPARATIVE STUDY BETWEEN PLEURAL FLUID CHOLESTEROL AND LIGHT'S CRITERIA IN DIFFERENTIATING EXUDATIVE AND TRANSUDATIVE PLEURAL EFFUSION

### ABSTRACT

**Background/Aims:** When the production and buildup of pleural fluid overwhelm its absorption, pleural effusion develops. Pleural effusions are classified as either exudates or transudates based on their pathogenesis. In order to treat the patient rationally, it is critical to make an appropriate etiological diagnosis. Discrimination between transudates and exudates using Light's criteria may require additional testing, however employing pleural fluid cholesterol makes diagnosis possible with only pleural fluid analysis.

**Materials and Methods:** This comparative study included 140 patients with pleural effusions. Pleural effusions were determined to be transudative or exudative on the basis of etiological diagnosis. The sensitivity, specificity, positive predictive value and negative predictive values of each test for the diagnosis of an exudative effusion were assessed.

**Results:** The pleural fluid cholesterol levels showed a sensitivity of 97.95%, a specificity of 95.23% ( $P < 0.0001$ ), a positive predictive value of 97.95% and a negative predictive value of 95.23%. The ROC curve analysis (receiver operating characteristic curve) of pleural fluid cholesterol with a cut-off of 45 mg/dl showed an area under curve of 0.986.

**Conclusions:** The pleural cholesterol level can be utilized as a biomarker to identify an exudative effusion and replace Light's criteria. It also eliminates the need of simultaneous blood sampling.

**Keywords:** Cholesterol, exudative effusion, pleural effusion.

### 1. INTRODUCTION

The parietal and visceral layers of the mesothelium are separated by a thin layer of fluid for lubrication that facilitates the movement of the two layers against each other. Therefore these cavities are not called true cavities, the presence of gas or fluid converts them into true cavities.

Collection of more than normal amount of fluid in a serous cavity is called effusion. It is classified on the basis of the location where it gets collected i.e. pericardial, pleural and peritoneal. The fluid getting collected in pleural cavity is called pleural effusion. Effusions can be classified into two types i.e. (1) transudative due to increased venous pressure and (2) exudative due to an increase in capillary permeability. It is important to differentiate between transudative and exudative causes of pleural effusion which helps in diagnosis as well as treatment<sup>[1]</sup>. In 1971 Light et al. devised a criteria

having high sensitivity and specificity of 99% and 98% respectively for differentiating transudative and exudative pleural effusion (ratio of pleural fluid protein by serum protein  $>0.5$ ; ratio of pleural fluid LDH by serum LDH  $>0.6$ ; pleural fluid LDH  $>2/3$ rd of upper limit of serum LDH)<sup>[2]</sup>. However other researchers were not able to reproduce similar results instead a specificity of only 70-86% was found using Light's criteria. Also 25% of patients with transudative effusion were wrongly tagged as exudative effusion. Transudative pleural effusions could have high protein in cases such as heart failure on diuretic therapy<sup>[3]</sup>. Pleural fluid cholesterol misclassifies fewer cases compared to Light's criteria<sup>[4]</sup>. From a meta-analysis done by Heffner et al. in the year 2002, pleural effusion was identified as exudative type by at least one of the following conditions.

- i. Pleural fluid protein  $>2.9$  gm/dL.
- ii. Pleural fluid cholesterol  $>45$  mg/dL (1.16 mmol/L).
- iii. Pleural fluid LDH  $>2/3$ rd of upper limit of serum<sup>[5]</sup>.

Pleural fluid cholesterol is hypothesised to be produced by degenerating cells and increased vascular permeability. Two hypotheses have been proposed, albeit the exact reason for the elevation in cholesterol levels in pleural exudates is unknown. According to the first, the pleural cells themselves produce cholesterol for their own needs<sup>[6]</sup> (extrahepatic synthesis of cholesterol is now known to be much greater than that was previously thought of, which depends on the metabolic needs of cells, and is in dynamic equilibrium with cholesterol supply by LDL and cholesterol removal by HDL) [6]. Furthermore, the degradation of leukocytes and erythrocytes raises the levels of cholesterol in the pleural cavity<sup>[7]</sup>. The second possible theory is that pleural cholesterol originates from plasma; plasma cholesterol is primarily bound to low density, high molecular weight lipoproteins (LDL), with the remaining serum cholesterol bound to very low density lipoproteins (VLDL) or HDL. Pleural exudate patients have increased pleural capillary permeability, which would allow plasma cholesterol to enter the pleural cavity. The choice of 45 mg/dL (1.16 mmol/L) as the cutoff value for pleural fluid cholesterol was made in order to avoid being ambiguous between transudates and exudates, and measurement of pleural cholesterol  $>45$  mg/dL (1.16 mmol/L) has been used to increase the accuracy of discriminating between transudative and exudative effusions<sup>[8]</sup>.

## **2. METHODOLOGY**

In this descriptive study 140 eligible patients with pleural effusion coming to Dhiraj General Hospital between the period of May 2021 to May 2022 were selected. The study was approved by ethics committee and informed consent was taken. Cholesterol estimation was done using Excel cholesterol kits analyzed on "ERBA EM 360 machine".

### **2.1 Collection of Fluids**

1. All aseptic precautions taken.
2. Samples collected in two vials— one in EDTA for cytology & another one in plain vial for biochemistry.
3. Samples were processed immediately.

### **2.2 Case Allocation**

In the present study 140 cases of pleural effusion were taken for a span of 12 months i.e. from May 2021 to May 2022. Various fluids samples were collected from OPD's and IPD's of Medical and Critical care units.

### **2.3 Principle**

Cholesterol ester hydrolase breaks down cholesterol esters to release free cholesterol molecules and fatty acids. There is pre-existing cholesterol that, along with the cholesterol created by the aforementioned reaction, is oxidized by cholesterol oxidase into cholestenone and hydrogen peroxide. Chromogen in the presence of H<sub>2</sub>O<sub>2</sub> and peroxidase is oxidized to a red colour complex that can be read at 520 nm. Unless placed in direct sunlight, this colour remains stable for two hours.

### **2.4 Procedure for Cholesterol Estimation**

- 1 tube was marked "blank" (B).
- One additional tube was marked as standard (S).

- The remaining tubes were given test (T) labels and codes depending on the number of pleural fluid samples.
- After bringing the cholesterol reagent to room temperature, 1 cc of reagent was poured into each tube. 10 microliters of distilled water was poured to tube B, and 10 microliters of standard cholesterol to tube S. Add 10 microliters of the relevant fluid sample's supernatant, labelled as T. All the tubes were kept at 37° C for 10 minutes. After calibrating using a standard, each reading was noted.

## 2.5 Statistical Analysis

The data was formulated in MS - Excel spread sheet, data summarized, frequencies & means calculated, further data analysis was performed.

## 2.6 Objectives of the study

To compare efficacy of pleural fluid cholesterol alone compared to Light's criteria for the diagnosis of exudative pleural effusion.

## 2.7 Inclusion Criteria

The inclusion criteria for the patients were as below:

1. Age  $\geq$  16 years,
2. Patients giving consent,
3. Patients with definite etiological diagnosis of causes leading to either transudative or exudative pleural effusion.

## 2.8 Exclusion Criteria

The exclusion criteria for the patients were as below:

1. Patients not willing to participate in the study,
2. Age < 16 years,
3. Patients without definite clinical diagnosis,
4. Patients previously diagnosed and already on treatment.

## 3. Results and Discussion:

A total of 140 patients with definite clinical diagnosis, eligible for the study, were included in which 71.42% (100) cases were exudates, and 28.57% (40) cases were transudates. It was seen that tubercular effusion was the most common pleural effusion in the study. It accounted for 49 out of 140 cases (35.0%). Carcinoma lung was the second most common cause accounting for 11.42% (16), followed by parapneumonic effusion 8.57% (12), empyema thoracis 6.42% (9), remaining % of exudative effusion was comprised of pancreatic pleural effusion (6), splenic abscess (5) and chylothorax (3). Transudates counted for 28.57% (40 cases).

**Table 1: Causes of exudative pleural effusion.**

Causes of exudative pleural effusion	Number	Percentage(%) of total patients
Tubercular effusion	49	35.00%
Neoplasia	16	11.42%
Parapneumonic effusion	12	08.57%
Empyema thoracic	09	06.42%
Others	14	10.00%

In the present study, it was found that mean plasma cholesterol level (mg/dL) was  $84.21 \pm 34.85$  for exudates and  $23.24 \pm 12.28$  for transudates.

It was seen that out of 140 cases (exudates 100 and transudates 40 according to etiological diagnosis), protein ratio (pleural fluid/ serum) identified 86 cases as exudates and 54 cases as transudates; LDH ratio identified 91 cases as exudates and 49 cases as transudates, while plasma cholesterol identified 98 cases as exudate, and 42 cases as transudates. It was seen that pleural fluid protein/serum protein ratio had a sensitivity of 84.44% and specificity of 80%; pleural LDH/serum LDH ratio has a sensitivity of 90.32% and specificity of 85.10%, and pleural fluid cholesterol with sensitivity

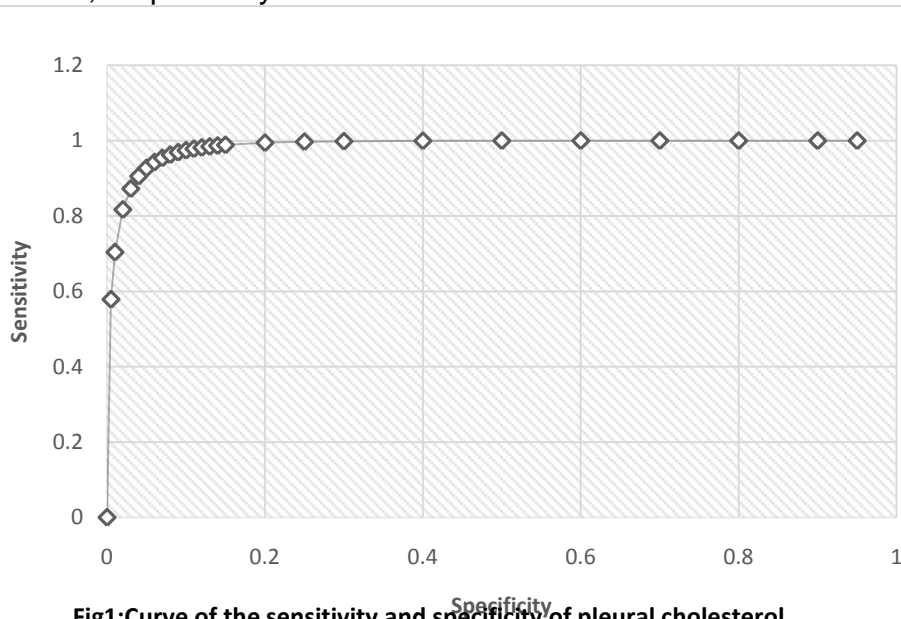
of 97.95% and specificity of 95.23% for differentiating exudative and transudative pleural effusion. All these parameters have a significant *P* value that is, <0.0001.

**Table 2: Comparison of various pleural fluid parameters.**

Parameters	Sensitivity	Specificity	PPV	NPV	<i>P</i> value
<b>Protein ratio (Pleural fluid/Serum)</b>	84.44%	80.00%	88.37%	74.07%	<0.0001
<b>LDH ratio (Pleural fluid/Serum)</b>	90.32%	85.10%	92.30%	81.63%	<0.0001
<b>Pleural fluid Cholesterol</b>	97.95%	95.23%	97.95%	95.23%	<0.0001

Receiver operating characteristic (ROC) analysis of pleural fluid cholesterol with a cut-off of 45 mg/dl showed a sensitivity (97.95%), specificity (95.23%) (*P* < 0.0001), area under curve 0.986, positive predictive value and negative predictive value of 97.95% and 95.23%, respectively.

Similar results were found in a study done by Rustogi et al who plotted a ROC analysis of pleural fluid cholesterol with a cut-off of 45 mg/dl which showed a sensitivity (98.18%), specificity (95.65%) (*P* < 0.0001), area under curve 0.969, positive predictive value and negative predictive value of 96.4% and 97.8%, respectively [9].



**Fig1: Curve of the sensitivity and specificity of pleural cholesterol.**

In a Receiver Operating Characteristic (ROC) curve, the true positive rate (sensitivity) is plotted as a function of the false positive rate (specificity) for the different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair that corresponds to a particular decision threshold. A test with perfect discrimination (no overlap between the two distributions) has a ROC curve that passes through the upper left corner (100% sensitivity and 100% specificity). Therefore, the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test. This ROC curve has an area of 0.986 (SD 0.0105), a confidence interval of 95% (0.965-1.007), and *p* < 0.0001.

## Discussion

Pleural fluid cholesterol is superior to Light's criteria for the differentiation of transudates and exudates and is less cumbersome in the clinical setting as it does not require a simultaneous blood sampling. Cut-off value of pleural fluid cholesterol for differentiating transudates and exudates should be 45 mg/dL [10]. In this study, a total of 140 patients, 40 with transudates and 100 with exudates, were considered according to the clinical diagnosis. The most frequent cause of pleural exudates is

tuberculosis followed by lung cancer which is similar to the result of a study done in Malaysia where there is high incidence of tuberculosis<sup>[11]</sup>. Measurement of pleural cholesterol >45 mg/dl has been used to improve the accuracy of differentiating transudative and exudative effusion<sup>[8]</sup>. In this study, the sensitivity, specificity, positive predictive value and negative predictive value of the pleural fluid cholesterol (cut-off >45 mg/dl) were 97.95, 95.23, 97.95 and 95.23%, respectively, for identifying exudates. Elevated cholesterol levels in exudates seem to be independent of serum levels<sup>[12]</sup>. The findings in a study done by Mehdi Kashmiri et al showed taking a value of pleural cholesterol >55 mg/dl and pleural/serum cholesterol >0.3 to define exudative effusion resulted in less erroneous classification with a sensitivity of 93%, a specificity of 100%, a positive predictive value (PPV) of 100% and an accuracy of 95.2%. Light's criteria yielded a sensitivity of 95%, a specificity of 95%, a PPV of 97.6% with an accuracy of 95.2%. Using pleural fluid cholesterol in differentiating exudate from transudate was especially useful in patients with congestive heart failure who received diuretics<sup>[13]</sup>.

#### **4. CONCLUSION**

Assessment using pleural fluid cholesterol can spare patients from having to pay for multiple tests used in Light's criteria to confirm an exudative pleural effusion, as well as from simultaneous blood sampling.

#### **CONSENT**

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

#### **ETHICAL APPROVAL**

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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