

Pleural Fluid Cholesterol: A Promising Marker to Differentiate Transudates and Exudates

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Abstract

Background: Categorization of pleural effusion into exudates and transudates is crucial for diagnosis and management. Light's criteria are the most commonly used method but has its own pitfalls. **Objective:** The objective is to study diagnostic value of pleural fluid cholesterol (pfCHOL) in differentiating transudative from exudative pleural effusions. **Patients and Methods:** A total of 101 subjects presenting with pleural effusion in the outpatient and emergency departments of a tertiary hospital were enrolled in the observational study. Pleural fluid protein, lactate dehydrogenase, adenosine deaminase, and pfCHOL were analyzed from pleural fluid samples obtained by diagnostic thoracentesis. Pleural fluid was classified into transudates and exudates based on the etiology (gold standard) against which Light's criteria and pfCHOL values were compared with appropriate statistical methods. **Results:** 74.26% were males. 46.53% were transudates as per the etiological diagnosis. According to the Light's criteria, 45.54% were transudates. With pfCHOL cut-off value of 1.16 mmol/L or 45 mg/dL, 44.55% were transudates. The transudates and exudates had a mean cholesterol level of 27.8 ± 7.84 mg/dl and 70.76 ± 22.35 mg/dL, respectively. A pfCHOL level of 45 mg/dl correctly identified 97.78% as transudates and 96.43% as exudates. Receiver operating characteristic analysis of pfCHOL with a cut-off of 45 mg/dl 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. **Conclusion:** pfCHOL is a less complex, cost-effective, reliable diagnostic marker and is as good as the Light's criteria to differentiate transudates and exudates.

Keywords: Exudates, pleural effusion, pleural fluid cholesterol, transudates

INTRODUCTION

Approximately one million people present with pleural effusion every year in outpatient department and the emergency department. The reported prevalence of pleural effusion is estimated to be 320/100,000. Pleural effusion is a presentation of many systemic and localized diseases, and the differential diagnosis is wide that it is often considered a diagnostic dilemma.^[1]

It can present either as a complication of diseases or as their presenting features and it is important to diagnose and treat it properly.^[2] The pleural space normally contains between 7 and 16 ml of fluid. When the rate at which the formation of pleural fluid is more than the rate of removal, accumulation of pleural fluid takes place.^[3]

Differentiating a transudative from exudative effusion is the first and the most critical step in the evaluation of the cause. Many criteria have been used till date for the same, but none of

them have been found to be satisfactory. Most commonly used is the Light's criteria which includes simultaneous measurement of the blood and pleural fluid protein and lactate dehydrogenase (LDH) levels. Pleural fluid is said to be an exudate if one of the subsequent 3 criteria is fulfilled:

- i. Value of protein in pleural fluid divided by serum protein >0.5
- ii. Value of LDH in pleural fluid divided by serum LDH making ratio >0.6
- iii. Value of pleural LDH $>2/3^{\text{rd}}$ of the higher limit of normal serum LDH.

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How to cite this article: Rustogi N, Gupta H. Pleural fluid cholesterol: A promising marker to differentiate transudates and exudates. *Indian J Respir Care* 2021;10:326-9.

Received: 07-03-2021

Revised: 08-08-2021

Accepted: 14-08-2021

Published: 13-09-2021

Access this article online

Quick Response Code:



Website:
www.ijrc.in

DOI:
10.4103/ijrc.ijrc_38_21

Light *et al.* in 1972 found that the criterion developed by him has a sensitivity and specificity of 99% and 98%, respectively, to distinguish transudate and exudate. However, other researchers were only able to reproduce specificities of 70%–86% after applying Light's criteria. The criterion has more specificity than sensitivity and typically identifies 98% of pleural exudates but misclassifies 25% of the transudates as exudates.^[4] As a result, patients who are labeled as exudate undergo irrelevant and risky invasive procedures, such as image-guided percutaneous pleural biopsy and thoracoscopic pleural biopsy.^[5] It has been seen that even after extensive workup, up to 20% of the cases remain undiagnosed.

Pleural fluid cholesterol (pfCHOL) is a recently studied parameter to differentiate transudates from exudates, which does not require simultaneous blood and other invasive workup for establishing the diagnosis. It is believed that the pleural cholesterol comes from dying cells and leakage from vessels that result from increased permeability. This study was conducted to evaluate the role of pfCHOL levels as a method for classifying transudates and exudates and its correlation with Light's criteria.

PATIENTS AND METHODS

The study was conducted in Department of Medicine at a tertiary care hospital. It is cross-sectional observational study conducted from November 2017 to March 2020. Permission was obtained from the institutional ethics committee to conduct the study. Study was conducted on 101 patients presented to the outpatient and emergency department of the hospital after taking proper informed consent.

Patients aged 18 years or older, of either gender presenting with newly diagnosed pleural effusion were included. Patients without definite clinical diagnosis, with pulmonary embolism, renal insufficiency, those previously diagnosed and already receiving treatment, traumatic pleural effusion, and chylothorax were excluded. Informed consent was taken from all patients included in the study.

After obtaining a detailed history and performing clinical examination, the following investigations were requested: complete hemogram, total protein, total cholesterol, LDH, renal function tests, and liver function tests, urine examination (routine and microscopy, culture sensitivity when needed), chest radiograph postero-anterior view, and electrocardiogram.

Diagnostic thoracentesis was done under aseptic conditions in every case with the help of ultrasonography chest to localize the fluid wherever necessary. All pleural fluid samples were tested for cell count, glucose, total protein, LDH, ADA, cholesterol [Table 1], malignant cytology, and gram stain. Investigations obtained as needed included ultrasonography chest, computed tomography chest, fine needle aspiration cytology to determine etiology and echocardiography.

Effusions associated with congestive heart failure and liver cirrhosis were classified as transudates and the rest

Table 1: Methods of estimation of biochemical parameters of pleural fluid and serum

Parameter	Method of estimation
Cholesterol	CHOD PAP method
Protein	Biuret method
ADA	Adazyme kit
LDH	Reflective spectrophotometric assay

ADA: Adenosine deaminase, LDH: Lactate dehydrogenase, CHOD PAP: Cholesterol oxidase/peroxidase aminophenazone

as exudates. Patients with renal disease and pulmonary embolism were excluded. The classification of pleural fluid into transudates and exudates based on the etiological diagnosis is considered as the gold standard against which the Light's criteria and pfCHOL (cut off 45 mg/dl) were compared.

Cholesterol estimation by CHOD-PAP method

The enzyme cholesterol esterase was used to hydrolyze the cholesterol esters present in the serum to free cholesterol and free fatty acids. The enzyme cholesterol oxidase in the presence of oxygen oxidizes the cholesterol to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically. It was done with the help of Randox kit on Vitros 5600 machine. Minimum concentration of cholesterol with an acceptable level of precision was determined to be 4.3 mg/dL.

Cholesterol ester + H₂O Cholesterol + free fatty acids

Cholesterol + O₂ Cholest-4-en-3-one + H₂O₂

2H₂O₂ + phenol + 4-AA Quinoneimine dye + 4H₂O

The intensity of color formed was proportional to the cholesterol concentration in the serum.

Statistical analysis

Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean ± standard deviation and median. Normality of data was tested by Kolmogorov–Smirnov test. If the normality was rejected, then nonparametric test was used. Quantitative variables were compared using independent t-test/Mann–Whitney test (when the data sets were not normally distributed) between the two groups. Qualitative variables were correlated using Chi-square test/Fisher's exact test. Receiver operating characteristic (ROC) curve was used to find cut-off point of parameters for predicting exudate. Comparison of ROC was performed to find any significant difference between area under the curve (AUC) of various parameters. A *P* < 0.05 was considered statistically significant. The data were entered in MS Excel spreadsheet, and analysis was done using the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp).

RESULTS

Of the 101 patients included, 74.26% were men. 53.85% of women had an exudative effusion and 46.15% had a transudative effusion. Among the men, 54.67% and 45.33% had exudative and transudative effusions, respectively. There was no difference in the gender distribution of transudative and exudative pleural effusion. We studied the frequencies according to various cut-offs for ages. For 20–30 years, it was 20.79%; for 30–40 years, 16.83%; for 41–50 years, 23.76%; for 51–60 years, 24.76%; and for 61–70 years, 13.86%. The highest percentage of patients was found to be in the age group of 41–50 years.

The percentage of transudates was 46.53% as against exudates of 53.46% as per the etiological diagnosis. The most common cause of transudate was chronic liver disease whereas the most common cause of exudate was tuberculosis [Figure 1]. According to the Light's criteria, 54.46% were found to be exudates and 45.54% transudates [Figure 2]. With the pfCHOL cut-off value of 1.16 mmol/L or 45 mg/dL, 55.45% were found to be exudates and 44.55% transudates [Figure 2]. The mean value of pfCHOL was 51.2 ± 27.56 and the exudates had a mean of 70.76 ± 22.35 and transudates a mean of 27.8 ± 7.84 mg/dL [Figure 3]. Among samples classified as exudates and transudates as per Light's criteria, a pfCHOL level of 45 mg/dl correctly identified 96.43% as exudates and 97.78% as transudates.

ROC analysis of pfCHol with a cut-off of 45 mg/dl in categorizing pleural effusions into exudate versus transudate showed a sensitivity of 98.18% and a specificity of 95.65% [Figure 4]. The results were statistically significant with a $P < 0.0001$, AUC of 0.969, positive predictive value (PPV) and negative predictive value of 96.4 and 97.8, respectively [Table 2].

DISCUSSION

Pleural cholesterol is thought to be derived from degenerating cells and vascular leakage from increased permeability. There are two possible explanations for the rise of cholesterol levels in pleural exudates.

According to the first explanation, the pleural cells synthesize cholesterol for their own needs. Extrahepatic synthesis of cholesterol is much greater than what was earlier believed and depends on the metabolic needs of cells. It is in dynamic equilibrium with cholesterol supply by

low-density lipoprotein (LDL) and cholesterol removal by high-density lipoprotein (HDL). Degeneration of leukocytes and erythrocytes leads to marked increase in the cholesterol levels as they contain tremendous amount of cholesterol.^[6]

Another explanation is that pleural cholesterol is derived from plasma. Around 70% of plasma cholesterol is bound to LDL, and the rest to HDL or very LDSs, and the increased permeability of pleural capillaries allows plasma cholesterol to enter the pleural cavity in exudative effusions.^[7]

Our study showed statistically significant values for pfCHOL value of 45 mg/dl in determining the nature of pleural effusion and was comparable to the Light's criteria.

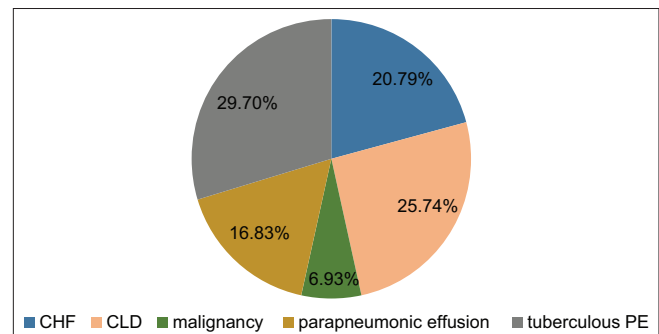


Figure 1: Showing percentage of transudates and exudates as per clinical diagnosis

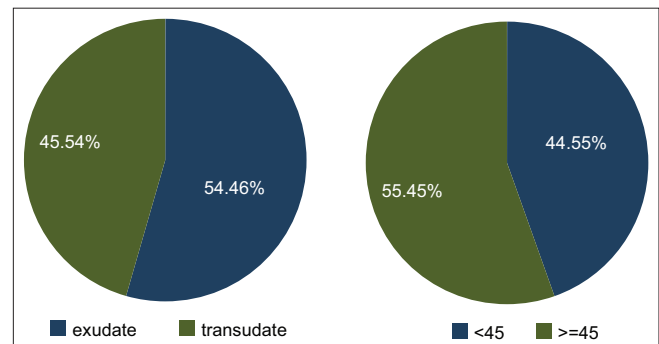


Figure 2: Showing percentage of exudates and transudates classified according to Light's criteria and pleural fluid cholesterol levels among total study population

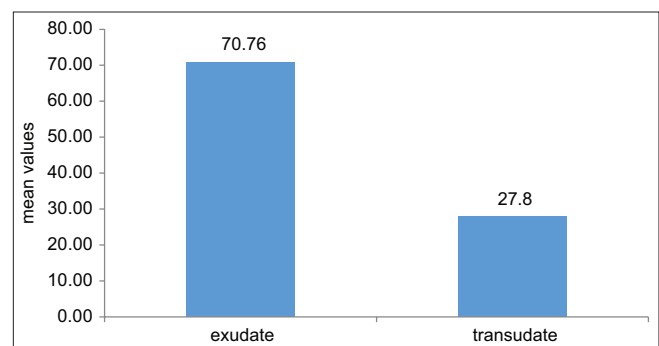


Figure 3: Showing mean values of pleural fluid cholesterol levels for transudates and exudates

Table 2: Sensitivity and specificity of pleural fluid cholesterol and parameters of lights criteria

Parameter	Sensitivity	Specificity	P	PPV	NPV
Cholesterol >45	98.18	95.65	<0.0001	96.4	97.8
Protein ratio	94.55	100	<0.0001	100	93.9
LDH ratio	98.18	95.65	<0.0001	96.4	97.8

LDH: Lactate dehydrogenase, PPV: Positive predictive value, NPV: Negative predictive value

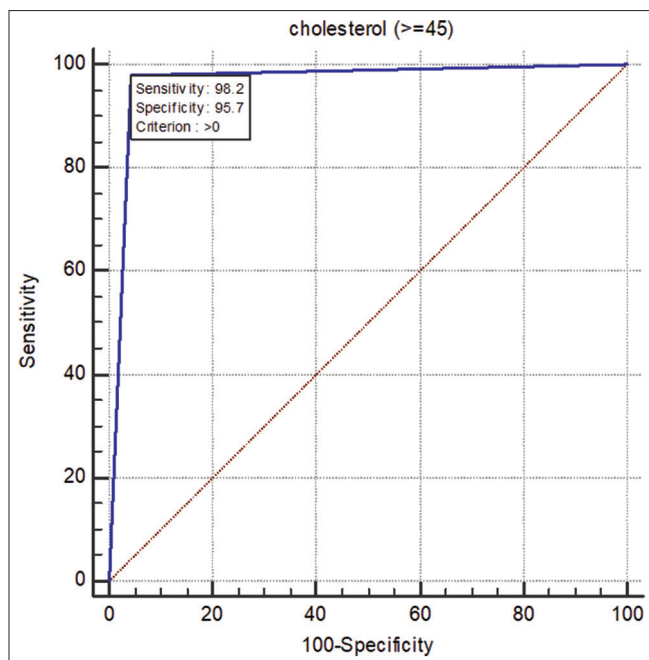


Figure 4: Receiver operating characteristic curve showing sensitivity of 98.2% and specificity of 95.7% at cholesterol cut-off of 45 mg/dL

Hamal *et al.* conducted a study and showed that pfCHOL has a better sensitivity, specificity, and PPV in differentiating transudates and exudates than the parameters of Light's criteria, thus concluding it as an efficient, easier, and a more cost-effective method and added that it should be routinely used in the evaluation of pleural effusion.^[8] Similar conclusions were drawn from other studies.^[9-12] Another study by Srinath *et al.* from India used a similar pfCHOL cut-off value of 45 mg/dL and concluded that the pfCHOL is better than Light's criteria in that it is less cumbersome as it does not require a simultaneous blood sampling.^[13]

With a total of 60 cases consisting of 43 exudative and 17 transudative effusions, Chakradhar Majhi *et al.* reported mean cholesterol levels of 64.2 ± 7.5 mg/dL in exudative effusions and 26.05 ± 8.01 mg/dL in transudates. pfCHOL was ≥ 55 mg/dL in 43 cases of exudates and < 55 mg/dL in 17 cases of transudate. The study thus concluded that a level of ≥ 55 mg/dL had a similar sensitivity and specificity to Light's criteria and can be used as a single important parameter to differentiate exudative from transudative pleural effusion.^[14]

Limitations

1. High pfCHOL in exudates was not correlated with serum albumin levels
2. Lipid profiles that might have effect on pfCHOL levels have also not been evaluated
3. Pleural fluid to serum cholesterol ratio was not calculated.

Future studies can probably evaluate these parameters.

CONCLUSION

pfCHOL is a promising marker with a high diagnostic accuracy due to high specificity and with a cut-off value of >45 mg/dL. It is comparable to Light's criteria in the differentiation of transudative and exudative pleural effusions. It has the added advantage of not requiring a simultaneous blood sample and that fewer parameters are tested, thus reducing the strain on laboratory resources. This is especially true in a country like India where financial constraints are huge.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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