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# Diagnostic value of pleural fluid cholesterol versus pleural fluid protein/ total serum protein ratio to differentiate Transudative pleural effusion from Exudative pleural effusion

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## Abstract

**Background:** Pleural effusion is a common condition in pulmonary medicine. Determining the cause of the pleural effusion is a difficult task. The distinction between an exudate and a transudate is the first and the most important step in the differential diagnosis of a pleural
effusion. Light's criterion have been previously used for classifying pleural effusion. There are multiple other biochemical markers which are available, the diagnostic accuracy of which is not well established and is a subject of debate.
**Aim:** To compare the diagnostic value of the pleural fluid cholesterol, ratio of Pleural Fluid Protein/ Total Serum Protein in differentiating the pleural fluid into transudate and exudate.
**Materials & Methods:** A total of 25 cases of pleural effusion due to different diseases were analysed using certain biochemical parameters like pleural fluid cholesterol, pleural fluid protein & its ratio with serum values were analysed.
**Result:** The pleural fluid protein, its ratio to serum protein and pleural fluid cholesterol had excellent diagnostic accuracy in differentiating exudative pleural effusions from transudative effusions.
**Conclusion**: The determination of pCHOL is of great value for distinguishing between pleural exudates and transudates and should be included in routine laboratory analysis of pleural effusion. The pleural fluid to serum protein ratio and pleural fluid cholesterol had excellent diagnostic accuracy in classifying the pleural fluid type.

**Keywords:** *Exudative Pleural effusion, Transudative pleural effusion, Pleural fluid cholesterol, ratio pleural fluid protein, serum protein.*

## Introduction

Pleural effusion is defined as the accumulation of serous fluid in pleural space (Crompton GK, Haslett C, Chilvers ER, 1999)[1]. Normally the pleural space contains less than 15ml of pleural fluid (Mohan Harsh, 1998)[2]. While evaluating a patient of pleural effusion, the first diagnostic step is to classify it as an exudative or transudative pleural effusion.

A transudative effusion occurs due to alteration in systemic factors that influence formation & absorption of pleural fluid, like decreased plasma colloid osmotic pressure or elevated hydrostatic pressure in systemic & pulmonary circulation. Common causes of transudative effusion are congestive heart failure, cirrhosis of liver, nephrotic syndrome & hypoprotienemia.

Exudative pleural effusion occurs due to alteration in local factors influencing formation & absorption of pleural fluid. It develops due to a disease of pleural surfaces. The two mechanisms that can cause exudative effusion are: increased permeability of pleural capillaries for proteins & lymphatic obstruction. Common causes of exudative pleural effusion are: Pulmonary tuberculosis, Pneumonia & Neoplasms. (Richard WL)[3]

Light et al[3] proposed biochemical parameters, i.e. protein & lactic dehydrogenase (LDH) to distinguish between transudative & exudative pleural effusion.

The criteria to diagnose an effusion to be exudative are;

1. Pleural fluid protein/serum protein> 0.5
2. Pleural Fluid LDH/ Serum LDH > 0.6
3. Pleural Fluid LDH >2/3 normal upper limit for serum

| Parameter | Exudative pleural fluid | Transudative pleural fluid |
|---|---|---|
| Protein (gm) / 100 ml | > 3 gm | <3gm |
| LDH level | >200 IU/L | <200 IU/ L |

In general, however, biochemical tests are not very helpful in a diagnostic sense (Anthony Seaton, 2000). The estimation of cholesterol in pleural fluid has been studied by various workers. The mechanism of rise in pleural fluid cholesterol is unknown. Two possible explanations have been put forward. One is that rise is due to cellular degeneration mainly of white & red blood cells & second is that cholesterol is derived from serum due to increased permeability of pleural capillaries in exudative pleural effusions (Ram & Jaya Sing, 1995)

Further studies by Hamm et al[5], Valdes L et al[6] & Costa et al[7] proved the importance of pleural fluid cholesterol in separation between exudative & transudative pleural effusions.

Living in a tropical country where pleural effusion is commonly encountered clinical condition, we need specific diagnostic parameters to differentiate between exudative & transudative effusions so that there is no extra burden of costly investigations for the patients. Therefore this study aims to compare pleural fluid cholesterol with the parameter pleural fluid protein/ serum protein ratio, with the aim to know usefulness of pleural fluid cholesterol for distinguishing between transudative & exudative pleural effusions.

## Aims & Objectives:

1. To estimate pleural fluid cholesterol level
2. To estimate pleural fluid protein, serum protein & pleural fluid protein/ serum protein ratio
3. To compare pleural fluid cholesterol level with pleural fluid protein/ serum protein level with regard to usefulness of pleural fluid cholesterol level for distinguishing between transudative & exudative pleural effusion.

## Materials and Methods

The study was conducted in the Departments of Medicine & Biochemistry, Govt Medical College & Hospital, Amritsar. Twenty five admitted cases with pleural effusion were studied prospectively. Informed consent was obtained from eligible patients for this study.

**Criterion for Diagnosis:** Diagnosis was done on the basis of History, Physical examination, X-ray chest, ultrasound chest for small pleural effusions. Besides pleural fluid cholesterol estimation & protein estimation, following routine investigations were done i.e Hb, TLC, DLC, ESR, Blood sugar, Blood Urea, serum creatinine, Urine Complete Examination, ECG.

Samples of pleural fluid were obtained by thoracocentesis & blood sample from antecubital vein was obtained within 48 hrs of admission. Both samples were immediately centrifuged and analysed for pleural fluid cholesterol, pleural fluid protein & serum protein.

### Examination of cholesterol:

Pleural fluid cholesterol was estimated by Zlatki's method modified by Zak[9].

### Principal:

Pleural fluid cholesterol is extracted quantitatively into an acetone alcohol mixture which also precipitates the protein. Part of extract is evaporated & is used for estimating cholesterol. Reagents used during the

procedure were: Acetone alcohol mixture 1:1 v/v, Glacial Acetic acid (Aldehyde Free), concentrated sulphuric acid, standard cholesterol solution (20, 40, 60, 80, 100 mg). 100 ml of glacial acetic acid was taken & pure dry cholesterol was dissolved in it & it was kept overnight in various cholesterol standard solutions.

1. Ferric chloride stock: 10 gm of ferric chloride & 0.6 ml of water was dissolved per 100 ml of Aldehyde free glacial acetic acid. The solution was discarded if deposits occurred.
2. King's Reagent (colour developing reagent): 0.1 ml ferric chloride solution was added into 9.9ml of concentrated sulphuric acid ($H_2SO_4$) containing in a dry 100ml measuring cylinder. A clear solution was prepared by shaking the mixture. The reagent was freshly prepared every time before use.

## Procedure:

Three long glass test tubes meant for cholesterol estimation were marked as Test (T), standard (S) & Blank (B) respectively.

## Test:

0.2 ml of pleural fluid specimen was pipette out into centrifuge tube and was added into 3.8 ml of acetone alcohol mixture to precipitate the proteins & was centrifuged. 2 ml supernatant was taken for estimation of total cholesterol in a long glass tube marked as "T". The tube was placed in boiling water bath for 2 minutes with constant shaking for evaporating the material to dryness. Care was taken not to char the material. Then 6 ml of glacial acetic acid was added.

## Standard:

6 ml of glacial acetic acid was added in a similar tube marked 'B'
4.0 ml of King's reagent was added to all test tubes marked T (Test), S (Standard), B (Blank) & was mixed well. Optical density (OD) of the purple colour produced at 540 nm after 20 min was noted.

## Calculation:

Pleural fluid cholesterol (mg/100ml)

$$= \frac{\text{O.D. of T- O.D. of B}}{\text{O.D. of S- O.D. of B}} \times \text{Concentration of Standard (mg/100 ml)}$$

## Standard for cholesterol:

A series of working standards of different strengths were prepared with 20, 40, 50, 60, 80 and 100 mg. 0.1 ml of each standard was added in glass test tubes, marked as S1, S2, S3, S4, S5 and so on, each containing 5.9 ml of glacial acetic acid.

## Estimation of Proteins:

Proteins were estimated by method of Rein et al, 1953[10]

Principal: Total serum proteins were estimated by treating serum with biuretic reagent. Proteins present in the sample form coloured complex with the cupric ions in the alkaline solution. The optical density of the coloured solution was noted at 540 nm and is compared with the absorbance of standard solution of unknown strength.

Reagents used in the procedure were:

1. Sulphate sulphite solution: 208 gm of anhydrous sodium sulphite and 70 gm of anhydrous sulphite solution was dissolved with stirring in900 ml of distilled water. Then 2 ml concentrated $H_2SO_4$ was added to make the volume 1 litre with water in a volumetric flask (278 gm/l)
2. Stock- Biuretic reagent: 45gm of sodium potassium tartrate (Rochelle salt) was dissolved in 400 ml of 0.2 N NaOH and then 15 gm of $CuSO_4$-$5H_2O$ was added, stirred continuously until the solution became complete. Then 5 gm of potassium iodide was added and made to a litre with 0.2 N NaOH.
3. Working Biuretic reagent: 200 ml of stock biuretic was diluted to 1 litre with 0.2 N NaOH ( 200 m Mol/2) containing 5 gm potassium iodide.
4. Solvent Ether (reagent grade)
5. Standard protein solution: 500 mg of bovine serum albumin was dissolved in 100 ml of normal saline (0.9% NaCl)

## Procedure:

7.5 ml of sulphate sulphite solution was added I a conical test tube and 0.5 ml of the sample was added. Stopper was put and this was mixed gently by inversion for 5-6 times. 2ml of mixture from this conical test tube was transferred into another tube marked 'T'.

Standard: 2.0 ml of standard protein (500 mg%) was taken in a test tube marked 'S'.

Blank: 2.0 ml of sulphate-sulphite solution was taken in the test tube marked 'B'.

To all the test tubes (T, S, B) 5.0 ml of biuretic agent was added & incubated in water bath at 37 degree for 10 minutes. Finally the optical density was read at 540 nm by setting the blank at zero.

Pleural fluid protein (gm/100ml)

$$= \frac{\text{O.D. of T- O.D. of B}}{\text{O.D. of S- O.D. of B}} \times \text{Concentration of standard (50 mg/100 ml)}$$

The values obtained were tabulated & analysed according to standard statistical methods.

## Results and Observations

All twenty five patients with pleural effusion were studied to distinguish between transudative & exudative pleural effusions with following parameters,

1. Pleural Fluid Cholesterol
2. Pleural fluid protein/ serum protein ratio

In the study group, 16 patients were males in the age group of 15-85 years & 9 patients were females in the age group 60-65 years. Mean age both for male & female patients were 52.16 years. This is statistically insignificant.

**Table I: Pleural fluid cholesterol levels in Transudative & Exudative Pleural Effusions**

| Transudative Pleural Effusion | | Exudative Pleural Effusion | |
|---|---|---|---|
| Pleural fluid cholesterol levels (mg %) | No. of cases | Pleural fluid cholesterol levels (mg%) | No. of cases |
| 21-30 | 2 (12.5%) | 40-60 | 1 (11.1%) |
| 31-40 | 4 (25%) | 61-80 | 6 (66.7%) |
| 41-50 | 10 (62.5%) | 81-90 | 2 (22.2%) |

Table I shows the Pleural fluid cholesterol levels in Transudative & Exudative Pleural Effusions. It can be seen that the maximum cases of transudative pleural effusions have pleural fluid cholesterol levels in the range of 41-50 mg % and maximum cases of exudative pleural effusions have pleural fluid cholesterol levels in the range of 61-80mg%.

**Table II: Pleural Fluid Protein Levels in Transudative & Exudative Pleural Effusions**

| Transudative Pleural Effusions | | Exudative Pleural Effusions | |
|---|---|---|---|
| Pleural Fluid protein Levels (mg%) | Number of cases | Pleural fluid protein Levels (mg%) | Number of cases |
| 0.1-1.0 | 2 (12.5%) | 2.1-3.0 | 2 (22.2%) |
| 1.1-2.0 | 4 (25%) | 3.1-4.0 | 3 (33.3%) |
| 2.1-3.0 | 4 (43.7%) | 4.1-5.0 | 3 (33.3%) |
| 3.1-4.0 | 31 (18.8%) | 5.1-6.0 | 1 (11.2%) |

Table II denotes that in maximum cases of transudative pleural effusions, pleural fluid protein levels are in the range of 2.1- 3.0 gm% and in maximum cases of exudative pleural fluid protein levels are in the range of 3.1-5.0 gm %.

**Table III : Pleural Fluid Protein (PPROT) Serum Protein (SPROT) & their ratio (P/SPROT) in Transudative & Exudative Pleural Effusion**

| Pleural Effusion | | PPROT gm/dl | SPROT gm/dl | P/SPROT gm/dl |
|---|---|---|---|---|
| Transudative Pleural Effusion | Range | 0.58-3.5 | 4.0-7.2 | 0.13-0.65 |
| | Mean | 2.32 | 5.77 | 0.40 |
| | S.D. | 0.87 | 0.93 | 0.15 |
| Exudative Pleural Effusion | Range | 2.9-5.7 | 5.0-7.8 | 0.52-0.79 |
| | Mean | 4.12 | 6.58 | 0.62 |
| | S.D. | 0.87 | 0.92 | 0.09 |
| PPROT | p<0.001 Highly significant | | | |
| P/S PROT | P<0.001 Highly significant | | | |

Table III shows the range, mean and standard deviation (SD) of pleural fluid protein & pleural fluid protein/serum protein ratio in transudative pleural effusions & exudative pleural effusions. In patients with transudative pleural effusions, protein values were less than the patient with exudative pleural effusions.

This table shows that patients with transudative pleural effusion had mean PPROT levels of 2.32 mg/dl whereas patients with exudative pleural effusions had mean PPROT levels of 4.12 mg/dl. The difference of PPROT mean value for the transudative group & its mean value for the exudative group was highly significant ($p<0.001$). Similarly patients with transudative pleural effusions had mean P/ SPROT ratio of 0.40 whereas patients with exudative pleural effusions had mean P/SPROT ratio of 0.62. This difference of P/SPROT mean value for transudative and its mean value for exudative group was also highly significant ($p<0.001$).

Table IV shows the range, mean & standard deviation of pleural fluid cholesterol (PCHOL) transudative pleural effusion & exudative pleural effusion.

**Table IV: Pleural Fluid Cholesterol Levels in Transudative & Exudative Pleural Fluids**

| | Transudative Pleural Effusions | Exudative Pleural Effusions |
|---|---|---|
| Range | 25-50 | 40-88 |
| Mean | 42.27 | 70.56 |
| SD | 7.17 | 14.32 |

The above table shows that in transudative pleural effusions pleural fluid cholesterol values are in range of 25-50 with a mean value of 42.27 and in exudative pleural effusions, pleural fluid cholesterol levels are in range of 40-88 with mean value of 70.56. The difference of pleural fluid cholesterol mean value for transudative group and for exudative pleural effusion group is highly significant ($p<0.001$).

**Table V: Number of misclassification in each group for Pleural fluid Protein/ Serum Protein Ratio & Pleural Fluid Cholesterol**

| Pleural Effusions | P/SPROT | PCHOL |
|---|---|---|
| Group I<br>Transudative Pleural Effusion | 3/16 | 0/16 |
| Group II<br>Tubercular Exudative Pleural Effusion | 0/7 | 1/7 |
| Group III<br>Non Tubercular exudative Pleural effusion | 0/2 | 0/2 |
| Total | 3/25 | 1/25 |
| Percentage | 12% | 4% |

Table V shows that misclassifications by pleural fluid cholesterol (PCHOL) is very low i.e. only one tubercular exudative pleural effusion was misclassified where as pleural fluid protein/serum protein ratio resulted in three misclassifications in transudative pleural effusions.

**Table VI: Specificity, Sensitivity, Positive predictive Value & Negative Predictive Value for the parameters Studied**

| Parameter | Specificity % | Sensitivity % | Positive Predictive Value | Negative Predictive Value |
|---|---|---|---|---|
| Pleural Fluid Cholesterol | 100% | 94.12% | 100% | 88.89% |
| Pleural Fluid protein/ Serum Protein Ratio | 75% | 100% | 81.25% | 100% |

The above table shows that pleural fluid cholesterol had sensitivity of 94.12% and specificity of 100% which is more than the pleural fluid protein/ serum protein ratio to differentiate transudative from exudative pleural effusion.

## Discussion

Pleural effusions occur during the course of a variety of diseases & represent one of the most common diagnostic problems encountered by the clinician. Frequently the cause of a pleural effusion is obvious for e.g. that associated with congestive heart failure. However even extensive diagnostic work up fail to reveal their etiology in as many as 10-20% of the cases.

The first step in determining the etiology of pleural effusion should be its classification either as a transudative or exudative pleural effusion, because transudative pleural effusions have few possible causes & do not require resorting to the diagnostic techniques that are necessary to distinguish among the many possible causes of exudative pleural effusions[6].

Light et al[3] concluded that a dividing line of 0.5 based on P/SPROT yielded somewhat better classification than the PPROT level of 3.0 gm and only one of the transudative pleural effusions in their series was incorrectly classified, but 10% of the exudative pleural effusions were still misclassified.

In present study, mean value for P/SPROT determined for transudative pleural effusions was 0.40 $\pm$ 0.15 and for exudative pleural effusion was 0.62$\pm$ 0.10. In the present study, using a dividing line of 0.5 resulted in misclassification of 3 transudative pleural effusions & no exudative pleural effusion was misclassified. Therefore P/ SPROT as a parameter with a dividing line of 0.5 to distinguish between transudative & exudative pleural effusions has obtained better results in our study.

Hamm et al[5] in their prospective study of 70 pleural effusions, demonstrated PCHOL levels of 60 mg per 100 ml as a simple & cost effective single test to distinguish between transudative & exudative pleural effusions and was observed superior to conventional measurement of protein level, LDH and Light's criteria.

Also, in the present study the mean value of pleural fluid cholesterol in transudative pleural effusions is 42.27 mg % whereas mean value of pleural fluid cholesterol in exudative pleural effusions is 70.56 mg %. Using a dividing line of 60 mg%, all the transudative pleural effusions were correctly classified whereas only one case of exudative pleural effusion was misclassified. The difference of pleural fluid cholesterol mean value for transudative & exudative pleural effusion group was highly significant ($p<0.001$). So the results of the present study are comparable to Hamm et al study[5].

Valdes et al[6] prospectively studied 253 pleural effusions and compared Light's criteria; PLDH, P/SLDH, P/SPROT ratio with PCHOL and P/SCHOL ratio with regard to their usefulness to distinguish between transudative & exudative pleural effusions. They reported that using a dividing line of 55 mg per 100 ml for PCHOL; none of the 65 transudative pleural effusions and 17 of 188 (9%) exudative pleural effusions i.e. 17 of total 253 (6.7%) pleural effusions was misclassified.

The mean value of PCHOL determined by Valdes et al[6] was 28.5 ± 12.8 for transudative pleural effusions & for malignant, tuberculous and miscellaneous exudative pleural effusions were 88.13 ± 30, 96.5 ± 28 and 88 ± 35.9 mg per 100 ml respectively. With PCHOL value of 55 mg/100 ml as dividing line between transudative & exudative pleural effusions, the sensitivity, specificity, positive predictive value, negative predictive value and efficiency determined by Valdes et al were 91%, 100%, 79% and 93.2% respectively. Valdes et al concluded determination of PCHOL and P/SCHOL both are highly effective in distinguishing between exudative & transudative pleural effusions.

In the present study, mean value of pleural fluid cholesterol level in transudative pleural effusion is 47.27 mg % and mean value of pleural fluid cholesterol level in exudative pleural fluid effusion is 69.49 mg %. The sensitivity, specificity, positive & negative predictive value is 94.12 %, 100%, 100% and 88.89% respectively. These values are comparable with the study by Valdes et al[6], Ram & Singh[10] & Gil Suay et al[11].

Vaz MA et al[12] suggested that Light's criteria represent the most acceptable method to separate transudative & exudative pleural effusions. To improve diagnostic accuracy, many biochemical markers have been proposed as alternatives to differentiate transudative & exudative pleural effusions. In most clinical studies, cholesterol has been shown to be as sensitive as the Light's criteria although it is less specific. The ideal cut off point of cholesterol to differentiate transudative pleural effusions and exudative pleural effusions is still unknown. Patel A.K., Choudhary S[13] in their study concluded that pleural fluid cholesterol and total serum proteins are simple,cost affective and useful parameters to differentiate transudative from exudative pleural effusion. Shen et al [14] in their study concluded that both pleural fluid cholesterol as well as P/S Cholesterol ratio are helpful for the diagnosis of pleural exudates. Hamal AB et al[15] in their study on 62 patients found a sensitivity of 97.7% and specificity of 100% in differentiating exudative and transudative pleural effusions.

In the present study, pleural fluid cholesterol has been shown to be more specific & sensitive than pleural fluid protein/ serum protein ratio (one of the Light's criteria). The ideal cut off point of pleural fluid cholesterol level to differentiate transudative from exudative pleural effusion is 60 mg%. So it is suggested that estimation of pleural fluid cholesterol should be a routine practice in pleural examination.

## Summary and Conclusions

The aim of the present study was to compare the diagnostic value of pleural fluid cholesterol versus pleural fluid protein/ serum protein to differentiate exudative from transudative pleural effusions. In this study, pleural fluid cholesterol was the single best criteria to distinguish transudative & exudative pleural effusions. So it is recommened to perform pleural fluid cholesterol levels in all cases of pleural effusions.

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