ABSTRACT: AIMS AND OBJECTIVES: Evaluation of pleural fluid cholinesterase level and comparison of study of pleural fluid cholinesterase levels and serum cholinesterase levels to differentiate transudates from exudates. Light's criteria were used to compare transudate from exudate.

MATERIALS AND METHODS: 56 patients of pleural effusion of different diseases like tuberculosis, malignancy, Para pneumonic effusion, congestive heart failure, nephrotic syndrome, pancreatitis were selected and studied for pleural cholinesterase levels and then serum cholinesterase levels.

RESULTS: In exudates it was observed that the mean PChe and P/S Ch were higher as compared to transudates (P < 0.001). the cutoff value for PChe is taken as 2000 IU/L for the diagnosis, with this level it was found that 4 % of transudates and 0 % of exudates were misclassified. when the cutoff value of 0.50 for P/S Che ratio was used the misclassification decreased to 2 % in exudates and 2% in transudates it was observed that Using Light’s criteria a sensitivity of 93 % and specificity of 85 % with positive predictive value (PP V) OF 90 % and negative predictive value (NPV) of 89 %.

CONCLUSION: Estimation of pleural cholinesterase levels and ratio of pleural cholinesterase to serum cholinesterase was more efficacious and specific compared to light’s criteria. Serum cholinesterase estimation is cost effective specific and more sensitive. It can be used routinely to differentiate transudates from exudates in pleural effusion of varied etiology.

KEYWORDS: Cholinesterase, Lactate Dehydrogenase, Light’s criteria.

INTRODUCTION: Pleural space lies between the lung and chest wall and normally it contains a very thin layer of fluid called pleural fluid. Pleural effusion is an accumulation of excess fluid in the pleural space that result when homeostatic forces that control the flow in and out of the space are disrupted. usually pleural effusion occurs when pleural fluid formation exceeds pleural fluid absorption. Pleural effusions evolve in the course of a variety of diseases. A correct diagnosis of the underlying disease is essential for rational management of the patient. In many case diagnosis may be established without difficulty using various laboratory investigations and radiological techniques. However, despite employment of extensive diagnostic procedures, the cause remains elusive in 10 to 20 % of all cases.

A primary diagnostic step is the identification of pleural effusion as either transudate or an exudate. Transudates are secondary to disease elevating hydrostatic pressures in the systemic or pulmonary circulation, or disease decreasing plasma colloid oncotic pressure. Exudates are due to disease which alters the permeability of the pleural surface or due to impaired lymphatic drainage.

Levels of pleural fluid protein and lactic acid dehydrogenase (LDH) are commonly analyzed to classify an effusion as an exudate or transudate. But the results have not always been satisfying. Lights criteria for pleural effusion has been widely used to differentiate between transudates and exudates.
However several reports 2-7 have shown that the Light et al criteria misclassify a large number of effusion, even up to 20 to 30% of transudates as exudates. Therefore several alternative parameters have been proposed e.g., pleural fluid cholesterol level and pleural fluid/serum cholesterol ratio. pleural fluid/serum bilirubin ratio, serum-pleural effusion albumin gradient, alkaline phosphatase vale and many others. But their superiority with respect to the criteria of Light et al remains doubtful on 1978. Eduardo et al2 and recently, in 2000, Janmeja et al6 have estimate pleural fluid cholinesterase and pleural fluid/serum cholinesterase ratio and found these to be more efficient for separating pleural transudates from exudates.

OBJECTIVES:
- To evaluate the usefulness of estimating the cholinesterase level in pleural fluid and pleural fluid to serum cholinesterase ratio for differentiating pleural transudates from exudates.
- To compare the diagnostic efficacy of these parameters with that of lights criteria.

METHODOLOGY: The study was conducted in Department of Medicine, Basaveshwara Teaching and general hospital, attached to Mahadevappa Rampure Medical College, Gulbarga. 50 patients with pleural effusion were selected for study between December 2006 to April 2008.

INCLUSION CRITERIA:
- Tubercular effusion was diagnosed by X-ray, pleural fluid and sputum AFB.
- Malignant effusion – malignant cells in the pleural fluid with or without histological evidence.
- CHF effusion as diagnosed by cardiomegaly on roentgenogram and echocardiography, presence of pulmonary congestion and absence of other lesions in the chest X-ray.
- Nephrotic syndrome as diagnosed by establishing proteinuria of > 3 gm/24 hours, oedema, hypoalbuminemia and hypercholesterolemia.
- Pancreatitis as diagnosed by history, serum amylase > 1000 u/ml and ultrasound abdomen.
- Pleural effusion due to other well determined cause.

EXCLUSION CRITERIA:
- Effusions of undetermined origin.
- Effusions with more than are possible cause.
- Empyemas.
- Hemothorax.
- Persons with history of exposure to organophosphorus compounds.

All the patients selected for the study were evaluated in detail, comprising of detailed history, clinical examination and relevant investigations.

Patients with clinical evidence of pleural effusion were first send for chest X –ray PA and lateral view and ultrasound thorax if required.

Following investigations were done on all the patients in the study Group:
1. Partial haemogram including ESR.
2. Urine albumin, sugar.
3. RBS.
4. Blood urea/creatinine.
5. Liver function tests.
6. Serum lactic dehydrogenase.
7. Serum cholinesterase.

Then the diagnostic thoracocentesis was performed taking great care not to let the fluid mix with blood.

**Pleural fluid was immediately sent for following Investigations:**

1. Pleural fluid cytology including malignant cells.
2. Proteins, sugar.
3. Lactic dehydrogenase.
4. Cholinesterase.

Effusions were individually classified as transudates or exudates after careful evaluation of all clinical data and investigation results. The criteria are analyzed for separation of transudative and exudative pleural effusions are as follows:

1. The criteria of Light et al, is in based on three parameters:
   a. Pleural fluid to serum proteins >0.5
   b. Pleural fluid to serum LDH > 0.6
   c. Pleural fluid LDH > 200 IU

   Exudative pleural effusion meets at least one of the following criteria, whereas transudative pleural effusion meets none.

2. Pleural fluid cholinesterase values > 2000 U/L are taken as exudates and those < 200 U/L are taken as transudate.
3. Pleural fluid to serum cholinesterase ratio: Ratio > 0.5 is taken as exudates and those < 0.5 are taken as transudate.

**ESTIMATION OF CHOLINESTERASE: PRINCIPLE: REACTIONS INVOLVED IN THE CHOLINESTERASE ASSAY ARE AS follows:** Cholinesterase hydrolyses Butyryl thiocholine to from thiocholine which reacts with 5.5- dithiobis-2-nitrobenzoic acid (DTNB) to yield coloured 5MNBA which is measured spectrophotometric ally at 410nm. Therefore, the rate of change in absorbance at 410nm is directly proportional to cholinesterase activity.

**REAGENT COMPOSITION:** CHOLINESTERASE R1.

**BUFFER SOLUTION:** Phosphate buffer. (pH 7.7) 50mmol/L, CHOLINESTERASE R2

**TABLETS:**

- 5, 5 DTNB 0.25mmol/L.
- Butyrylthocholine 7mmol/L.

**NORMAL RANGE:** It is recommended that each laboratory establish its own reference vale.
SERUM: 4659 – 14443 U/L.

REAGENT PREPARATION: Dissolve one tablet R2 in one vial of Reagent R1. Wait for 10 Minutes for complete dissolution.

REAGENT STORAGE AND STABILITY: Dry regent is stored at 2-8º C, and is stable till the expiry date or reagent. Reconstituted regent is stable for 2 hours at 2-8º C.

PROCEDURE:
- 20µ of sample (10µl of serum/pleural fluid + 10µl of saline solution) is added to 3000µl of the working reagent. Test is performed on semi-automated biochemistry analyzer.
- This cholinesterase assay is a kinetic test with a delay time of 60 sec, followed by two readings of absorbance again at 60 sec interval each. Average difference in.

    Absorbent/min (ΔOD/ min) is obtained and multiplied by the factor 22710 to obtain the results. This whole process was programmed in to the analyzer and result was automatically obtained. Test was performed at 37º C.

STATISTICAL ANALYSIS: Different formulas used for result analysis are:

1. Student unpaired ‘t’ test was used to compare various parameters used in the study. This test in a parametric test described by W.S. Gossett whose pen name was ‘Student’. It is used to comparison of small number of samples is less than 30.

\[
\begin{align*}
\text{Sensitivity} & = \frac{TP}{TP+FN} \\
\text{Sensitivity} & = \frac{TP}{TP+FP} \\
\text{Positive predictive value} & = \frac{TP}{TP+FP} \\
\text{Negative predictive value} & = \frac{TP}{TP+FP} \\
\text{Efficacy} & = \frac{TP+TN}{TP+FN+TN+FP}
\end{align*}
\]

TP-True positive, TN-True Negative, FP- False Positive, FN-False Negative
RESULTS

Following observation were made after studying 50 cases of pleural effusion admitted to Basaveshwara Teaching and General Hospital, Gulbarga between Dec 2012 to April 2014.

Table No. 1
Showing Sex Distribution

| Sex     | No. of Cases | Percentage |
|---------|--------------|------------|
| Males   | 33           | 66         |
| Females | 17           | 34         |

Total 50 cases were included in this study, 33 were males and 17 females.

Table No. 2
Table showing Age Distribution

| Age  | Male | Female |
|------|------|--------|
| 11-20| 04   | 03     |
| 21-30| 05   | 03     |
| 31-40| 01   | 02     |
| 41-50| 04   | 00     |
| 51-60| 10   | 04     |
| 61-70| 06   | 05     |
| > 70 | 03   | 00     |
| Type       | No. of Cases | Percentage |
|------------|--------------|------------|
| TRANSUDATES| 20           | 40         |
| a) CCF     | 18           | 36         |
| b) Nephrotic syndrome | 01   | 02         |
| c) Cirrhosis| 01           | 02         |
| EXUDATE    | 30           | 60         |
| a) Malignant| 04           | 08         |
| b) Non Malignant| 26 | 52         |
| • Tubercular | 17           | 34         |
| • Pneumonia | 07           | 14         |
| • CRF      | 01           | 02         |
| • Post CABG| 01           | 02         |

Table 3: Showing Types of Effusion and their Percentage

| Investigation                      | Transudate | Exudate |
|------------------------------------|------------|---------|
| Pleural                            | <3gm       | >3gm    |
| PF/S* Protein ratio                | <0.5       | >0.5    |
| Pleural LDH                        | <200 IU    | >200 IU |
| PF/S* LDH ratio                    | <0.6       | >0.6    |
| Pleural fluid cholinesterase       | <2000 IU   | >2000 IU|

Table 4: Showing Cutoff Values of Different Parameters of Differentiate between Transudate and Exudate

Cut off values of pleural fluid proteins, PF/S protein ratio, pleural fluid LDH, PF/S LDH ratio are fixed according to the Light's Criteria. Pleural fluid cholinesterase value of 2000 IU and PF/S cholinesterase ratio 0.50 are based on mean and standard deviation (Mean-2SD) with clinical features and positivity of the test.
Investigation | Transudate (n=20) | Exudate (n=30) | t-value* | p-value**
--- | --- | --- | --- | ---
Protein | Pleural fluid | 2.27± 0.77 | 4.38±0.98 | 8.51 | <0.001
 | PF/S * ratio | 0.26±0.17 | 0.66±0.12 | 9.30 | <0.001
LDH | Pleural fluid | 346.2±234.8 | 1247.43±994 | 4.78 | <0.001
 | PF/S ratio | 0.72±0.68 | 1.92±1.42 | 4.10 | <0.001
Cholinesterase | Pleural fluid | 1111±362 | 5433±1587 | 14.36 | <0.001
 | PF/S ratio | 0.18±0.11 | 0.64±0.06 | 16.15 | <0.001

Table 5: Showing Comparison of Different Parameters between Transudates and Exudates

*t value – Student t-test.
PF/S- Pleural fluid to serum ratio.
** P-value <0.5 significant
<0.001 Highly significant

Investigations | Transudates | Exudate
--- | --- | ---
 | No. of Cases (n=20) | Percentage | No. of Cases (n=30) | Percentage
Light's criteria | 03 | 06 | 02 | 04
Pleural fluid Cholinesterase | 02 | 04 | 02 | 00
PF/S cholinesterase ratio | 01 | 02 | 01 | 02

Table 6: Showing Number and Percentage of Misclassifications as Transudates and Exudates by using different Parameters

The misclassification of transudates and exudates were less pleural fluid to serum cholinesterase ration, compared to all other parameters and Light's criteria. But even pleural fluid to serum cholinesterase ratio misclassified one each case of transudate and exudate.

Criteria’s | CCF (n=18) | Nephrotic Syndrome (n=1) | Cirrhosis (m=01) | Total (n=20) | Percentage
--- | --- | --- | --- | --- | ---
Light’s Criteria | 03 | 00 | 00 | 03 | 06
Pleural fluid Cholinesterase | 02 | 00 | 00 | 02 | 04
PF/S cholinesterase ratio | 01 | 00 | 0 | 01 | 02

Table 7: Number and Percentage of Misclassified Transudates

Light’s criteria misclassified 3 transudates, while PF/S cholinesterase 1 transudates.
Criteria’s | PTB | Pneu. | CRF | Post CABG | Malig. | Others | Total | Percentage
--- | --- | --- | --- | --- | --- | --- | --- | ---
Light’s Criteria | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 4
Pleural fluid Cholinesterase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0
PF/S cholinesterase ratio | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2

Table 8: Number and Percentage of Misclassified Exudates

Light’s criteria misclassified 2 exudates PF/S cholinesterase ratio misclassified only 1 exudate and pleural fluid cholinesterase misclassified none.

| Investigation | Sensitivity | Specificity | PPV % | NPV % | Efficiency |
| --- | --- | --- | --- | --- | --- |
| Light’s Criteria | 93 | 85 | 90 | 89 | 90 |
| PF Cholinesterase | 100 | 90 | 94 | 100 | 97 |
| PF/S Cholinesterase | 97 | 95 | 97 | 95 | 96 |

Table 9: Showing Diagnostic Validity of various Parameters
DISCUSSION: Pleural effusion occurs in a large variety of conditions, but correct diagnosis of underlying diseases is essential for rationale management of pleural effusion. Early evidence of the transudative or exudative nature of a pleural effusion may be of considerable clinical value and is often used as a basis for further diagnostic procedures and therapeutic considerations. Many tests we used to differentiate transudate from exudates by specific gravity, cell count and presence or absence of clotting of fluids. But no single chemical tests or series of tests has yet proved to be completely reliable.

In their study in 1972, Light et al used pleural and serum levels of proteins and LDH to establish criteria for segregation transudates from exudates with a sensitivity and specificity of 99% and 98% respectively. Recently however, several prospective studies 2-7 were unable to reproduce the excellent results obtained by light et al.

In most of those studies sensitivity for exudates remained high, about 95% but specificity did not surpass 78%. Therefore several alternative parameters have been proposed e.g. plural fluid/ serum cholesterol ratio 2. pleural fluid/ serum bilirubin ratio 3, serum pleural effusion albumin gradient4, alkaline phosphatese5 and many others.

In 1978, Carbrer et al analyzed the cholinesterase activity in pleural effusions of diverse causes and found a significant difference in the average level of cholinesterase. Between transudates and exudates. This fact later became the basis for using cholinesterase levels for differentiating transudative from exudative plural effusions.

In the present study, by using pleural to serum cholinesterase ratio, we could accurately classify 96% of pleural effusion cases. Out of the 50 cases evaluated, 1 each case of transudate and exudates were misclassified. By using pleural fluid cholesterase. Level 2 cases of transudates were misclassified. By using Light’s criteria 10 % of cases were misclassified.

Eduardo et al in 1996, conducted study on 153 patients with pleural effusion to differentiate transudate and exudates. They compared Lights criteria with pleural fluid cholesterol, pleural fluid to serum cholesterol, pleural fluid cholinesterase and pleural fluid to serum cholinesterease ratio.

RESULTS WERE AS FOLLOWING:
- Misclassification rates by Lights criteria was 7.8 %
- Misclassification rates by pleural fluid cholesterol level with cut off value of mg/dl was 7.8 %
- Misclassification rates by Pleural fluid to serum cholesterol with cut off value 0.30 was 6.5 %
- Misclassification rates by pleural fluid cholinesterease with cut off values of 1140 for Group A and Group B was 8.5%.

Misclassification rates by pleural fluid to serum cholinesterase ratio with cut of value 0.23% was only 1.38%. These criteria correctly classified 98.77% of 153 pleural effusion patients.

| Criteria Used          | Study of Eduardo et al | Present Study |
|------------------------|------------------------|--------------|
|                        | % of Misclassification | % of Misclassification |
| Light’s criteria       | 7.8                    | 10           |
| PF Cholinesterase      | 8.5                    | 4            |
| PF/S Cholinesterase    | 1.38                   | 4            |

Table 10: Showing Comparison of Misclassification of Pleural Effusion by Eduardo et al study and Present Study using Various Parameters
In the present study, the misclassifications using PF to serum cholinesterase ratio is higher than the study by Eduardo et al. But both the studies shows that compared to Light et al criteria, pleural fluid to serum cholinesterase ratio is a better parameter to separate transudate from exudates. Jasmeja et al in 2000 studied 50 cases of pleural effusion with different etiology using PF/S cholinesterase as criteria.

THEY MADE TWO GROUPS IN STUDY POPULATION:

Group A: Compromise 10 case each of PTB, malignancy and para pneumonic plural effusion total of 30 cases.

Group B: Compromised 10 pleural effusions due to CHF and 10 cases due to nephritic syndrome total of 20 cases.

By using 0.5 as the cutoff value for PF/S cholinesterase ratio they were able to classify 98% of effusion accurately, by taking 1600 IU of pleural fluid cholinesterase as cutoff value they misclassified 3 cases of effusion, and by using Light’s criteria 5 cases were misclassified.

| Criteria Used      | Study of Eduardo et al | Present Study |
|--------------------|-------------------------|---------------|
| Light's criteria   | 10%                     | 10%           |
| PF Cholinesterase  | 06%                     | 04%           |
| PF/S Cholinesterase| 02%                     | 04%           |

Table 11: Showing Comparison of Misclassification of Pleural Effusion by Janmeja et al and Present Study using Various Parameters

In the present study the misclassifications using PF to serum cholinesterase is higher than the study of Janmeja et al. Misclassifications using Light’s criteria is also high with the present study. But both studies show that PF to serum cholinesterase is a better parameter to separate transudates from exudates.

The present study and the study by Eduardo et al shows that number of misclassification is higher using only pleural fluid cholinesterase, compared to pleural fluid to serum cholinesterase ratio. While present study, using PF cholinesterase misclassified 6% of pleural effusions, Eduardo et al and Janmeja et al misclassified 8.5% and 6% cases each respectively. This could be attributed to the fact that, since the cholinesterase is synthesized in the liver, the levels can be influenced by different disorders like hepatitis, cirrhosis, acute infections, pulmonary embolism chronic renal failure and after surgical procedures.

In the present study the cut of value of pleural fluid cholinesterase and pleural fluid to serum cholinesterase ratio is 2000IU and 0.5 respectively. Pleural fluid cholinesterase >2000 and Pleural fluid to cholinesterase ration> 0.5 is taken as exudates and the value <2000 a ratio <0.5 is taken as transudates. This cut of value is based on Mean and standard deviation (Mean-2 SD) with clinical features and positively of the test. For transudate the value is 1111 ± 362 and for exudates the value is 5433± 1587. The difference between transudates and exudates are statistically highly significant (p<0.001).
The present study and various other studies, brings to the light the fact that Light’s et al. criteria misclassify transudate more than the exudates. In the present study using Light’s criteria the misclassification ratio were 6% for transudates 4% for exudates.

| Study                  | Transudates % | Exudates % |
|------------------------|---------------|------------|
| Eduardo et al          | 25            | 2.5        |
| Janmeja et al          | 15            | 6.6        |
| Bernard J. Roth et al  | 8             | 0          |
| Present study          | 6             | 4          |

Table 12: Showing Difference Between Misclassification to Transudates and Exudates by Various Studies using Light’s Criteria

In the present study the sensitivity and specificity using pleural fluid to serum cholinesterase ratio is 97 % and 95% respectively while the corresponding values using Light’s Criteria is 93% and 85% respectively. This shows that sensitivity and specificity of pleural fluid to serum cholinesterase, to separate transudate from exudates is higher than that of Light’s criteria. Also the efficiency of pleural fluid to serum cholinesterase ratio to separate transudate is 96% while that of Light’s criteria is only 90%.

The most analyzed parameters in the recent past to separate transudative from exudative effusions have been pleural fluid cholesterol level and pleural fluid to serum cholesterol ratio. In the study of Valdes et al the misclassification rates were 6.8 by pleural fluid cholesterol, while pleural fluid to serum cholesterol ratio misclassified 6.6% cases. Meisel et al in his study using pleural fluid to serum bilirubin ratio as parameters to separate transudate and exudates misclassified 19% Pleural effusion. Roth et al analyzed the serum-albumin gradient in their study of 59 patients correctly classified all the transudates and 3% of the 41 exudates but other authors could not reproduce similar satisfactory results. Tahaoglou et al found the level of alkaline phosphate lower in transudates but misclassification rate was 6.5%.

The results of our study compared with the parameters used by others suggest that the pleural fluid to serum cholinesterase ratio criterion is the accurate parameter for differentiation transudative and exudative pleural effusions.

CONCLUSION:

- Misclassification is present with all parameters used.
- Number of misclassifications using PF/S cholinesterase ratio and pleural fluid cholinesterase level are less compared to other parameter used.
- Sensitivity and specificity of PF/S cholinesterase ratio and pleural fluid cholinesterase level as diagnostic parameter to differentiate transudates and exudates are higher than all other parameters used in the study.
- The numbers of misclassification are more with the Light's criteria than PF/S cholinesterase ratio.
- Hence, the pleural fluid to serum cholinesterase ratio and pleural fluid cholinesterase level are the reliable method in separating pleural transudates from exudates. If further studies with larger study group confirm our results, cholinesterase ratio and pleural fluid cholinesterase level could be used as the first step in diagnostic study of pleural effusion.
SUMMARY:

- Fifty patients with pleural effusion were studied over a period of 17 months from December 2006 to April 2008 in Basaveshwara Teaching and General Hospital, Gulbarga.
- Out of 50 cases, 33 were males and 17 females.
- Study group comprised of mainly two types of pleural effusion.
  
  **Group A:** transudative pleural effusion - 20 cases.
  
  **Group B:** Exudative pleural effusion - 30 cases.
  
- Pleural fluid cholinesterase value of > 2000 IU and PF/S cholinesterase ratio of > 0.50 was taken as exudates and corresponding values of < 2000 IU and < 0.50 was taking as transudate.
- Misclassifications are present with all parameters used.
- The number of misclassifications are more with the Light's criteria than the pleural fluid cholinesterase and PF/S cholinesterase ratio, and pleural fluid cholinesterase level as diagnostic parameter to separate transudative from exudative pleural effusion are higher than the Light’s criteria.

LIST OF ABBREVIATIONS USED:

| Abbreviation | Description |
|--------------|-------------|
| PF           | Pleural Fluid |
| PF/S         | Pleural Fluid to Serum |
| SChe         | Serum Cholinesterase |
| PChe         | Pleural Fluid Cholinesterase |
| Che          | Cholinesterase |
| LDH          | Lactate Dehydrogenase |
| TB           | Tuberculosis |
| AFB          | Acid Fast Bacilli |
| ADA          | Adenosine Deaminase |
| CT           | Computed Tomography |

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