Evaluation of Cholinesterase to Differentiate Pleural Exudates and Transudates

Manju Sharma*, KB Gupta**, Kirori M Goyal**, Nitya Nand***

Abstract
Aims and Objectives: The present study was undertaken to evaluate the usefulness of pleural fluid cholinesterase (PChE) level in pleural fluid and its ratio to serum cholinesterase (P/SChE) in order to differentiate transudates and exudates and to compare their diagnostic efficacy with the Light’s criteria.

Material and Methods: A total of 110 patients of pleural effusion of diverse etiology were studied. Eighty patients were of exudative pleural effusion of tubercular, malignant or parapneumonic origin and 30 patients were of transudative effusion. Cholinesterase was estimated in the pleural fluid and serum in all the patients.

Results: The mean PChE and P/S ChE were significantly higher in exudates as compared to transudates (p < 0.001). P/S ChE was 0.79 ± 0.45 and 0.14 ± 0.11 in exudates and transudates, respectively. When a cut-off value of 469 IU/L for PChE was taken for the diagnosis, it was found that 10% of exudates and 2.5% of transudates were misclassified. However percentage of misclassification decreased to 1.25% in exudates and 3.3% in transudates when the cut-off value of 0.24 for P/S ChE ratio was used. Using Light’s criteria, a sensitivity of 91.25% and specificity of 90% with positive predictive value (PPV) of 96.05% and negative predictive value (NPV) of 79.42% was observed. However using P/S ChE, the PPV was 98.75% and NPV was 96.67%.

Conclusions: The estimation of PChE and P/SChE ratio had better discriminatory capacity than Light’s criteria. It is cost effective and more specific, therefore its routine estimation is recommended.

INTRODUCTION
A correct diagnosis of the underlying disease is essential for rationale management of pleural effusion. Transudative pleural effusion is caused by a limited number of diseases, whereas the exudate effusion requires more extensive diagnostic investigations. Therefore, the first step is to classify them as transudate or exudate effusion, even if this differentiation does not contribute to the etiological diagnosis. Many criteria have been used to distinguish them, but none of them has been found to be satisfactory. It was found that Light’s criteria misclassified large number of effusions. Later on some modifications were made in Light’s criteria by many researchers. However, all these parameters also classified some pleural effusions falsely. Cholinesterase (ChE) activity is significantly inhibited in the presence of untreated pulmonary tuberculosis and its activity remains stable in anyone individual. Therefore, measurement of its activity may serve as a useful diagnostic too. Cabrer et al estimated ChE activity in pleural effusions of diverse etiologies and concluded that there exists differences in the activity of ChE and it was possible to differentiate transudate and exudate. In 1996, Eduardo et al concluded that pleural fluid to serum cholinesterase ratio was the most accurate criterion for separating pleural fluid transudate and exudate and suggested that it should be used as the first step in the diagnosis of pleural effusions, if further studies confirmed their results. Garcia and Padilla confirmed the importance of estimation of ChE activity in the diagnosis. However, literature on such a work is scanty and no Indian work is available on the subject. It was in this context that the present study was planned to evaluate the diagnostic efficacy of the estimation of cholinesterase level in pleural fluid and pleural fluid to serum cholinesterase ratio for classifying transudative and exudative pleural effusions in the light of best analyzed criteria of Light et al and others.
**Material and Methods**

The present study was conducted on 110 adult subjects of pleural effusion, which were divided into two groups. Group I, consisted of 80 patients with exudative pleural effusion including 25 patients of tuberculosis, 40 cases of malignancy and 15 cases of parapneumonic effusion. Group II, consisted of 30 patients with transudative pleural effusion, which included 15 patients of nephrotic syndrome and 15 cases of congestive heart failure. Etiological diagnosis of pleural effusion was confirmed by accepted criteria.4,10

Exclusion criteria included: (i) patients having pleural effusion with more than one etiology or having hepatic disease, (ii) patients using oral contraceptive pills, anticancer drugs, monoamine oxidase inhibitors, neostigmine, chlorpromazine chloride, cyclophosphamide and pregnancy as all these conditions are known to affect/alter the ChE activity.11 The diagnosis of transudative and exudative pleural fluid was made by estimation of ChE and the ratio of pleural fluid to serum cholinesterase as well as by Light’s criteria and the results of the two were compared.

Protein levels were estimated in serum and pleural fluid by the Biuret method.12 Lactate dehydrogenase (LDH) activity was measured in serum and pleural fluid by decrease in absorbance of NADH at 340 nmm.13 Cholinesterase estimation was done according to procedure of Dietz et al.14 based on Ellman reactions15 by kinetic colorimetric method using Boehringer Manhaeim/Autopak cholinesterase kit and it was expressed as units/liter.

**Statistical analysis**

Students ‘t’ test was applied to statistical analysis. The usefulness of biochemical parameters (cholinesterase and Light’s criteria) for identifying exudates was evaluated using Bayesian method in terms of sensitivity (TP/PN+FN), specitivity (TN/TN+FN), positive predictive value (TP/TP+FP) and efficiency (TP+TN/TP+TN+FP+FN), where TP is the number of true positive diagnosis, TN the number of true negative diagnosis, FP the number of false positive diagnosis and FN is the number of false negative diagnosis.

**Results**

Differences between the mean values of pleural fluid protein (P protein) and ratio of pleural fluid to serum protein (P/S protein) for transudative and exudative groups (Table 1) were highly significant (p < 0.001). Mean pleural LDH was 318.07 ± 123.34 and 155.60 ± 88.76 IU/L in exudative and transudative pleural effusions, respectively.

Six cases in transudative and 12 cases of exudative effusion were misclassified by P Protein level, where as five cases in transudate and 10 cases in exudate were misclassified on basis of P/S Protein ratio. P/S LDH misclassified five cases in transudative and seven cases of exudative pleural effusion. Pleural fluid cholinesterase (PChE) misclassified three cases of transudative and two cases of exudative pleural effusion, while ratio of pleural fluid to serum cholinesterase (P/SChE) falsely classified only one case in transudative and two cases in exudative effusion. Misclassification of pleural effusion was observed more in transudative than in exudative pleural effusions (Table 2). Further maximum misclassification was seen when P protein parameter was used for the diagnosis and it was minimum when P/SChE estimation was employed.

We used cut-off value of 469 U/L for P ChE (which is 1/40000 of mean value for normal volunteers) for identification of exudate.

**Table 1**: Various parameters studied in exudative and transudative pleural effusions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exudate</th>
<th>Transudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant</td>
<td>Tubercular</td>
</tr>
<tr>
<td>P protein (g%)</td>
<td>3.59 ± 0.95</td>
<td>4.13 ± 1.30</td>
</tr>
<tr>
<td>P/S protein</td>
<td>0.63 ± 0.17</td>
<td>0.67 ± 0.20</td>
</tr>
<tr>
<td>P LDH (IU/L)</td>
<td>295.32 ± 97.6</td>
<td>354.08 ± 152.51</td>
</tr>
<tr>
<td>P/S LDH</td>
<td>0.84 ± 0.25</td>
<td>0.96 ± 0.33</td>
</tr>
<tr>
<td>P ChE</td>
<td>1123.07 ± 441.23</td>
<td>1050.96 ± 448.66</td>
</tr>
<tr>
<td>P/S ChE</td>
<td>0.86 ± 0.49</td>
<td>0.75 ± 0.46</td>
</tr>
</tbody>
</table>


**Table 2**: Misclassification of exudative and transudative effusions using various parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Transudate (n=30)</th>
<th>Exudate (n=80)</th>
<th>Total (n=110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P protein</td>
<td>6 20.00</td>
<td>8 20.00</td>
<td>3 12</td>
</tr>
<tr>
<td>P/S protein</td>
<td>5 16.6</td>
<td>6 15.00</td>
<td>3 12</td>
</tr>
<tr>
<td>P LDH</td>
<td>7 23.3</td>
<td>3 7.5</td>
<td>3 12</td>
</tr>
<tr>
<td>P/S LDH</td>
<td>5 16.06</td>
<td>4 10.00</td>
<td>2 8</td>
</tr>
<tr>
<td>P ChE</td>
<td>3 10.00</td>
<td>1 2.5</td>
<td>1 4</td>
</tr>
<tr>
<td>P/S ChE</td>
<td>1 3.3</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

In the past, transudates were separated from exudates by the specific gravity, cell count and presence or absence of clotting of fluid. In 1972, Light et al. developed a criterion for the diagnostic separation of transudates from exudates. Thereafter it was found that Light’s criteria misclassified large number of effusions which may lead to unwarranted invasive procedures in 10-20% of cases, the cause of pleural effusion remains elusive.

Cholinesterase is found in plasma and most tissues. It is synthesized in the liver and its levels are remarkably constant in well-fed healthy individual. Measurements of serum ChE activity can serve as sensitive measures of the synthetic capacity of the liver. The cholinesterase activity may vary widely from individual to individual, but it tends to remain fairly constant in any one individual. Although there are number of clinical conditions where its activity is altered, however, its use for differentiating exudative and transudative effusion has been more promising. On the basis of P/S ChE ratio, Eduardo et al. concluded that it was the most accurate criteria for differentiating transudates and exudates.

In the present study, the mean serum cholinesterase was significantly lower in exudative effusions as compared to the transudative effusion (p < 0.001). However the mean levels of cholinesterase in different subgroups of exudative effusion were not statistically different. When we used a cut-off value of 0.24 for P/S ChE ratio we were able to classify 98.19% of effusion correctly with PPV 98.75% and NPV 96.67%. Misclassification was observed in 3.3% of transudates and 1.25% of exudates with an overall misclassification of 1.81% only. In the present study, by using Light’s criteria, we found sensitivity of 91.25%, specificity of 90% with positive predictive value of 96.05% and negative predictive value of 79.42%. The efficiency was to the tune of 90.90%, which is much lower than P/SChE ratio (Table 3).

### DISCUSSION

Many biochemical parameters like pleural fluid cholesterol, bilirubin, albumin, alkaline phosphatase, adenosine deaminase, malondialdehyde (MDA) and their ratio with serum values have been used to differentiate the type of pleural effusion. Cholesterol is constantly present in pleural effusion and its high level is observed in tubercular effusion. However, extraordinarily high level of cholesterol in pleural fluid is independent of its value in the serum. Gupta et al. studied pleural fluid cholesterol and its ratio with the serum cholesterol in 70 cases of pleural effusion and found that the estimation was not only cost effective but also extremely useful parameter to distinguish transudate from exudate. However, despite deployment of extensive diagnostic procedures in 10-20% of cases, the cause of pleural effusion remains elusive.

### REFERENCES

4. Vives M, Porcel JM, Vicente de Vera MC, Ribelles E, Rubio M. A study of Light’s criteria and possible modifications for


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