EFFICACY OF PLEURAL FLUID ADENOSINE DEAMINASE AND C-REACTIVE PROTEIN LEVELS IN EARLY DIFFERENTIAL DIAGNOSIS OF PLEURAL EFFUSION.

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ABSTRACT: - CONTEXT: Pleural effusion occurs secondary to various diseases. Common causes of exudative effusion are tuberculosis, bacterial pneumonia, and malignancy. Transudative effusion is due to systemic diseases like cardiac failure, cirrhosis of liver. Conventional methods of diagnosis may not be able to establish the cause of pleural effusion. Early diagnosis and management reduces the morbidity and mortality. AIM: The objective of the study is to estimate pleural fluid Adenosine Deaminase (ADA) and C - reactive protein (CRP) levels and to evaluate their efficacy in differential diagnosis of transudative and exudative, tuberculous and non tuberculous and inflammatory and non inflammatory effusions. MATERIAL AND METHODS: Fifty two patients of pleural effusion were investigated and divided into four groups based on diagnosis. Group I, II, III and IV had 24 cases of tuberculous effusion, 13 cases of transudative effusion, 08 cases of malignant effusion and 07 cases of parapneumonic effusion respectively. Pleural fluid was analyzed for ADA (Guisti and Galanti’s method) and CRP (turbidometric immunoassay).STATISTICAL ANALYSIS: The statistical analysis was done using unpaired student’t’ test and p value < 0.05 was considered statistically significant. RESULTS: In the present study pleural fluid ADA revealed highly significant increase in tuberculous effusion than non tuberculous effusions (p <0.001) and also when compared with non tuberculous subgroups, transudative effusion (p < 0.001), malignant effusion (p<0.001), and PPE (p<0.01). ADA levels at a cutoff value of 40U/L, showed sensitivity, specificity, positive predictive value and negative predictive value of 91.67%, 89.3%, 88% & 92.6% respectively in tuberculous effusion. Pleural fluid CRP levels in parapneumonic effusion were significantly higher compared to other types of effusions (p<0.001). Significantly higher levels of CRP were seen in exudative effusion compared to transudative effusion (p<0.001) and in inflammatory effusion compared to non inflammatory effusion (p<0.001).
CONCLUSIONS: Pleural fluid ADA is sensitive test in discriminating tuberculous and non tuberculous effusions. Pleural fluid CRP levels distinguish transudative from exudative effusion, inflammatory from non inflammatory effusion. ADA and CRP assays are rapid, minimally invasive and cost effective and measurement of these two parameters increases the efficacy of diagnosing pleural effusion.

KEY WORDS: Adenosine deaminase, C - reactive protein, Pleural effusion.

INTRODUCTION: Pleural effusion (PE) is a common complication associated with number of diseases. Accumulation of pleural fluid may be due to various causes such as increased pleural membrane permeability, increased capillary pressure, decreased oncotic pressure and lymphatic obstruction. Transudative pleural effusions occur when systemic factors which effect the formation and absorption of pleural fluid are altered. Exudative pleural effusion occur secondary to local diseases.

The leading causes of pleural effusion are left ventricular failure, cirrhosis, bacterial pneumonia, malignancy, viral infections and pulmonary embolism. Tuberculous (TB) pleurisy is the major cause of pleural effusion.

In India, TB is the commonest cause of pulmonary disease. About 5 lakh people suffering from TB die every year with pulmonary TB often associated with pleural effusion. Tuberculous pleural effusion is the second most common cause of extra pulmonary TB secondary to only lymphoid TB.

Analysis of pleural fluid (PF) is an important tool in correctly diagnosing the etiology of pleural effusion. Conventional methods of diagnosis may not be able to establish the cause of pleural effusion or give an early diagnosis. The diagnosis of tuberculous pleural effusion is difficult as tubercle bacilli is rarely found from thoracocentesis and pleural lavage and other non invasive traditional tools of diagnosis have low sensitivity and specificity.

Adenosine deaminase (ADA), an enzyme of purine salvage and catabolic pathway deaminates adenosine and deoxyadenosine to form inosine and deoxy inosine respectively. ADA is involved in the proliferation and differentiation of T lymphocytes. TB pleurisy is a result of delayed hypersensitivity reaction in response to mycobacterium antigen. Measurement of ADA in PF has been widely used in the differential diagnosis of lymphocytic exudative pleural effusion as high values have been found in tuberculous pleural effusion. ADA test is inexpensive, minimally invasive, rapid, has high sensitivity and specificity for diagnosis of tuberculous pleural effusion. However, ADA levels have also shown to be increased in some other conditions like rheumatoid arthritis, empyema, mesothelioma, bronchial carcinoma, fungal infections etc.

C - reactive protein (CRP) is an acute phase protein synthesized by hepatocytes and used as marker of inflammation and tissue injury. CRP is thought to assist in complement binding to foreign and damaged cells and increases the phagocytosis by macrophages. It plays an important role in innate immunity against infection.

In many studies, CRP levels have been found to be higher in exudates when compared to transudates. In exudates higher levels have been found in parapneumonic pleural effusions and TB pleural effusions.
The aim of the present study was to evaluate the efficiency of pleural fluid ADA and CRP levels in differential diagnosis of tuberculous and non tuberculous effusions, transudative and exudative effusion and between inflammatory and non inflammatory pleural effusions.

MATERIAL AND METHODS: The study group consisted of 52 patients with pleural effusion of different etiology, age ranging from 20 to 85 years. Informed consent was taken and the study was approved by ethical and research committee of the institution.

The patients were categorized into four groups based on diagnosis.

GROUP I: Tuberculous pleural effusion (24 cases)
GROUP II: Transudative pleural effusion (13 cases)
GROUP III: Malignant pleural effusion (08 cases)
GROUP IV: Parapneumonic effusion (PPE) (07 cases)

The diagnosis of etiology of pleural effusion was done based on clinical presentation, radiological examination and laboratory investigations.

Diagnosis of TB pleural effusion was done when positive for any one of the following test: presence of tubercle bacilli in smear or in culture of pleural fluid, caseating granulomas in histopathological study, radiological findings consistent with TB, response to antitubercular treatment. Malignant pleural effusion was diagnosed when PF cytology showed evidence of malignancy and or neoplastic pleural tissue in pleural biopsy. PPE was diagnosed when patient had fever, pulmonary infiltrates in chest X-Ray and who responded to antibiotic treatment. Transudative and exudative pleural effusion were distinguished based on protein and LDH levels in pleural fluid and serum as per Light's criteria.²

Pleural tap was done in all cases and the pleural fluid was analysed for sugar, protein, lactate dehydrogenase (LDH), ADA and CRP levels.

Pleural fluid sugar, protein and LDH were analysed using Erba reagent kits on EM 200 analyzer.

The study parameter ADA in pleural fluid was measured using the kit purchased from Tulip Diagnostic (P) Ltd on Erba chem 5 plus analyzer. The kit provides the reagents for Guisti and Galanti’s method of ADA estimation.¹⁰ The assay is based on deamination of adenosine by ADA to form ammonia and inosine. The ammonia reacts with phenol and hypochlorite in an alkaline medium in presence of sodium nitroprusside to form blue indophenol complex, the intensity of which is directly proportional to ADA levels.

The other study parameter CRP was measured by turbidometric immunoassay using the kit purchased from Tulip Diagnostic (P) Ltd, on Erba chem 5 plus analyzer. The test is based on the principle of agglutination reaction between latex reagent and CRP to form insoluble complex with resultant increase in turbidity measured at 546nm wavelength.

STATISTICAL ANALYSIS: The results were expressed as mean ± standard deviation (SD). The statistical analysis was done using unpaired student 't' test and probability value (P) value < 0.05 was considered statistically significant. Sensitivity, specificity, positive predictive value and negative predictive value were calculated.
RESULTS: Fifty two cases of pleural effusion were investigated. The subjects were divided into four groups as shown in Table 1/Fig 1.

Pleural fluid was analyzed for Protein, Sugar, LDH, ADA and CRP levels and the results are shown in Table 2/Fig 2.

Pleural fluid ADA levels were highest in tuberculous effusion and the difference in ADA levels between tuberculous and other effusion was statistically highly significant (transudative effusion p<0.001, malignant effusion p<0.001 and PPE p<0.01) (Table 3/Fig 3).

ADA levels were compared between tuberculous effusion and non tuberculous effusion. Statistically significant increase was seen in tuberculous effusion than non tuberculous effusion (p<0.001) (Table 4/Fig 4).

Among tuberculous effusion only 02 cases had ADA levels less than 40U/L and among non tubercular group only 03 cases had ADA levels greater than 40 U/L (Table 4/Fig 4). ADA at a cut off value of 40U/L for diagnosis of tubercular effusion, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was 91.67%, 89.3%, 88% and 92.6% respectively.

Pleural fluid CRP levels were compared in different groups of pleural effusion. PPE had significantly higher values than other types of pleural effusion (p<0.001) (Table 3/Fig 3). Pleural fluid CRP levels in exudative effusion were significantly higher than transudative effusion (p<0.001). There was also significant increase in inflammatory effusion when compared to non inflammatory effusion (p<0.001) (Table 5/Fig 5).

All patients with PPE had CRP levels greater than 6mg/dl and all patients of tuberculous effusion had CRP levels greater than 2mg/dl. Except one case of mesothelioma, all malignant effusion and transudative effusion had CRP levels less than 2mg/dl.

DISCUSSION: Pleural effusion occurs secondary to either systemic causes or disease of pleura. Conventional non invasive diagnostic methods are not always accurate in establishing the diagnosis of pleural effusion. Analysis of pleural fluid yields important information in early differential diagnosis of pleural effusion. Standard workup analysis of pleural fluid includes differentiating whether pleural fluid is transudative or exudative. For many years the most accepted criteria for discriminating transudative from exudative pleural effusion is Light's criteria. However Light's criteria may differentiate certain transudative effusion as exudative effusion. The most important cause of transudative pleural effusion is cardiac failure. TB is the leading cause of preventable morbidity and mortality from an infective agent and tuberculous effusion is important treatable cause of exudative pleural effusion. Other common causes of exudative effusions are malignancy, parapneumonic pleural effusions, connective tissue disorders, fungal infections etc.

Various biological markers have been investigated in the diagnosis of pleural effusion. Among these pleural fluid ADA, CRP, interferon γ, cytokines, interleukins, tumour markers, vascular endothelial growth factor have been found to be of value in the differential diagnosis of pleural effusion. Nevertheless many of these markers have limited value, either because of low sensitivity & specificity or high cost.

The diagnosis of tuberculous pleural effusion is difficult because of low sensitivity and specificity of various non invasive tools like acid fast bacilli staining, culture of pleural tap and...
tuberculin skin testing. Diagnosis increases to 96.2% with pleural biopsy but the disadvantage of this technique is its invasiveness.4

Another important rapid technique for diagnosis of tuberculous pleural effusion is polymerase chain reaction (PCR) with high sensitivity and specificity. PCR is positive in 100% of culture positive cases of tuberculous effusion and in only 30-60% of culture negative effusion. The disadvantage of PCR is high cost, skilled technology and risk of DNA contamination. Hence routine use of PCR is not feasible in the diagnosis of tuberculous pleural effusion.5

ADA is considered as indicator of cell mediated immunity and is found in T lymphocytes and macrophages.12 Many studies have found utility of pleural fluid ADA levels with good sensitivity and specificity in diagnosis of tuberculous effusion.

In the present study pleural fluid ADA levels were compared between the four groups of pleural effusion. The values were highest in tuberculous effusion and lowest in transudative effusion. The difference in ADA levels between tuberculous and non tuberculous effusion was statistically significant (p<0.001). Also the difference in ADA levels between tuberculous effusion and transudative effusion, malignant effusion and PPE were statistically significant (p<0.001, p<0.001 and p<0.01). Similar report was also seen by BK Gupta et al.4

In the present study two of tuberculous pleural effusion cases had ADA values less than 40 U/L. A similar report was given by Motoki S et al where they found 12% of the tuberculous pleurisy patients having ADA levels less than 50U/L and out of this 6% of them had less than 35U/L.3

The ADA levels at a cutoff value of 40 U/L indicated tubercular pleurisy with a sensitivity of 90-100% and specificity of 89-100%.6 Wipa R et al reported 80% sensitivity and 80.5% specificity at a cutoff value of 48U/L in diagnosing tuberculous effusion.12 Burgess LJ et al showed 90% and 89% sensitivity and specificity for identification of TB pleurisy at a cutoff value of 50U/L.13

In the present study at a cutoff value of 40U/L, the sensitivity, specificity, PPV and NPV of pleural fluid ADA levels in diagnosis of tuberculous pleural effusion was 91.67%, 89.3%, 88% and 92.6% respectively.

However Rafael L. cautioned the use of pleural fluid ADA assay as an alternative to biopsy and culture, but should rather be considered as a screening test to guide further diagnostic management.6 High levels of ADA have also been reported in many other conditions including malignant effusion, rheumatoid arthritis, systemic lupus erythematosis, empyema and fungal infections.1 In the present study two empyema cases of non tubercular origin showed very high values of ADA.

In a report by E Garcia Pachon et al, a patient with mesothelioma had high ADA levels (73U/L) and CRP concentration of 8.9mg/L and they reviewed that elevated levels of ADA are seen in approximately a third of mesothelioma patients.14 In the present study a patient diagnosed as mesothelioma had ADA level of 61U/L and CRP level was 0.8mg%. A study by SK Verma et al also found ADA levels in malignant PE ranging from 18.5 to 87.6U/L.15

In another study by D Jimenez Castro et al on ADA levels in non tuberculous pleural effusion, a negative predictive value of 99% was reported for diagnosis of non tuberculous pleural effusion and the ratio of ADA1/ADA2 correctly classified all the cases as non tuberculous pleural effusion.16 ADA exists in two isoenzyme forms, ADA1 is expressed in all cells where as ADA2 is found only in monocytes.6
C-reactive protein is another sensitive marker in distinguishing the diagnosis of pleural effusion. It is widely used as a maker of inflammation and tissue injury. CRP levels have been found higher in benign than malignant pleural effusion. High pleural fluid CRP levels have been reported in tuberculous pleural effusion and PPE.

In the present study CRP levels were lowest in transudative effusion when compared to exudative effusion which was highly significant (p<0.001). A high significant increase was seen in inflammatory pleural effusion (tuberculous effusion and PPE) when compared to non inflammatory effusion (transudative and malignant effusion) (p<0.001). Tuberculous pleural effusion had high CRP levels when compared to transudative and malignant pleural effusions which were highly significant (p<0.001). But the highest values were found in PPE (p<0.001) which was highly significant when compared with transudative effusion, tuberculous effusion and malignant effusion (p<0.001).

Yilmaz UT et al, reported high levels of CRP in exudates when compared to transudates and high levels in parapneumonic pleural effusions when compared to other types of exudative pleural effusions and also reported high sensitivity (93.7%), specificity (76.5%) and PPV of 98.4% at a cutoff value of 30mg/L. A similar finding was also reported by Castano-Vidrialesa JL et al, and they reported good sensitivity (82%), specificity (87.5%) and PPV (95.5%) in diagnosis of exudative pleural effusion.

Hoda Abu-Youssef et al showed high values of CRP in exudative pleural effusions when compared to transudative pleural effusions. However in their study tuberculous effusion had statistically higher CRP levels when compared to malignant pleural effusions and parapneumonic pleural effusions. A similar finding was reported by EG Pachon et al it was concluded that a CRP level <20mg/L suggested malignant pleural effusion and a value >45mg/L virtually excluded the diagnosis of malignant pleural effusion.

Pleural fluid CRP levels have also been useful in discriminating uncomplicated parapneumonic pleural effusions from complicated parapneumonic pleural effusions and empyema. High levels of CRP have been found in complicated PPE and very high levels are seen in empyema cases.

Daniil ZD et al, evaluated multiple biomarkers in discriminating pleural effusion. They concluded the combination of ADA and CRP levels might be sufficient in discriminating the three different groups of pleural effusion, tubercular, malignant and PPE. In the present study, in most cases of tuberculous pleural effusion the ADA levels were >40U/L and CRP levels >2mg/dl, in PPE the ADA levels were <40 U/L (except empyema cases) and CRP levels >6mg/dl, where as in both malignant and transudative pleural effusions the ADA levels were <40U/L (except a mesothelioma case) and CRP levels <2mg/dl. The present study is in accordance with findings of Daniil ZD et al.

CONCLUSION: Both pleural fluid ADA and CRP testing are minimally invasive, inexpensive and efficacious method of differentiating pleural effusion. These markers together increase the diagnostic efficacy in discriminating the tuberculous, transudative, PPE and malignant pleural effusion. However larger studies are required to evaluate usefulness of CRP in discriminating tuberculous effusion and PPE.

REFERENCES:


**TABLE 1/FIG 1**: Distribution of cases in different groups of pleural effusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>No of cases</th>
<th>Mean Age in years</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculous pleural effusion</td>
<td>24</td>
<td>42.95</td>
</tr>
<tr>
<td>II</td>
<td>Transudative pleural effusion</td>
<td>13</td>
<td>58.30</td>
</tr>
<tr>
<td>III</td>
<td>Malignant pleural effusion</td>
<td>08</td>
<td>53.75</td>
</tr>
<tr>
<td>IV</td>
<td>Parapneumonic pleural effusion</td>
<td>07</td>
<td>40.71</td>
</tr>
<tr>
<td>Total</td>
<td>All groups</td>
<td>52</td>
<td>47.57</td>
</tr>
</tbody>
</table>

**TABLE 2/FIG 2**: Pleural fluid protein, sugar and LDH* levels in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Protein (gm%) Mean ±SD</th>
<th>Sugar (mg %) Mean ±SD</th>
<th>LDH* (U/L) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculosis pleural effusion</td>
<td>4.9±0.92</td>
<td>59.1±11.24</td>
<td>139±51.6</td>
</tr>
<tr>
<td>II</td>
<td>Transudative pleural effusion</td>
<td>2.1±0.39</td>
<td>67.8±13.41</td>
<td>85.5±27.9</td>
</tr>
<tr>
<td>III</td>
<td>Malignant pleural effusion</td>
<td>4.7±0.41</td>
<td>44.3±11.26</td>
<td>253.5±139.7</td>
</tr>
<tr>
<td>IV</td>
<td>Parapneumonic pleural effusion</td>
<td>5.1±0.79</td>
<td>48.0±7.83</td>
<td>180.2±78.8</td>
</tr>
</tbody>
</table>

* Lactate Dehydrogenase

**TABLE 3/FIG 3**: Pleural fluid adenosine deaminase and C-reactive protein levels in different types of pleural effusions

| Group | Diagnosis                          | ADA* (U/L) Mean ±SD | P value | CRP|| (mg/dl) Mean ±SD | P value |
|-------|------------------------------------|---------------------|---------|-----------------------|---------|
| I     | Tuberculous pleural effusion       | 130.66±82.10        | -       | 3.21±0.81             | 0.001** |
| II    | Transudative pleural effusion      | 18.34±6.02          | 0.001†  | 0.80±0.42             | 0.001†† |
| III   | Malignant pleural effusion         | 31.46±15.29         | 0.001‡  | 1.21±1.05             | 0.001‡‡ |
| IV    | Parapneumonic pleural effusion     | 56±47.56            | 0.01§   | 7.32±0.98             | -       |

* Adenosine deaminase
† p value between tuberculous effusion and transudative effusion
‡ p value between tuberculous effusion and malignant effusion
§ p value between tuberculous effusion and parapneumonic effusion
|| C- reactive protein
** p value between parapneumonic effusion and tuberculous effusion
†† p value between parapneumonic effusion and transudative effusion
‡‡ p value between parapneumonic effusion and malignant effusion

**TABLE 4/FIG 4**: Pleural fluid ADA* levels in tubercular and non tubercular pleural effusion.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ADA* (U/L)</th>
<th>No of patients with ADA*</th>
<th>No of patients with ADA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>greater than 40U/L</td>
<td>less than 40U/L</td>
</tr>
<tr>
<td>Tuberculous effusion</td>
<td>130.66±82.10</td>
<td>22 (91.66%)</td>
<td>02(8.33%)</td>
</tr>
<tr>
<td>Non tuberculous effusion</td>
<td>31.50±28.60</td>
<td>03 (10.71%)</td>
<td>25 (89.28%)</td>
</tr>
</tbody>
</table>

* Adenosine deaminase

**TABLE 5/FIG 5**: Pleural fluid CRP* levels between transudative & exudative pleural effusion and between inflammatory & non inflammatory pleural effusions.

<table>
<thead>
<tr>
<th>CRP* mg/dl</th>
<th>Transudative PE†</th>
<th>Exudative PE†</th>
<th>Inflammatory PE†</th>
<th>Non inflammatory PE†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Tuberculous, PPE‡ and malignant)</td>
<td>(Tuberculous and PPE‡)</td>
<td>(Transudative and malignant)</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>0.80±0.42</td>
<td>3.54±2.14</td>
<td>4.13±1.93</td>
<td>0.95±0.73</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* C-reactive protein
† Pleural effusion
‡ Parapneumonic pleural effusion