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Study of Diagnostic Importance of Adenosine Deaminase (ADA) Level in Pleural Effusions

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Abstract

Introduction: Pleural effusion is the abnormal accumulation of fluid in the pleural space. TB is the most common cause of pleural effusion worldwide (30-60%). The pleural fluid activity of adenosine deaminase (ADA) is one of the best, providing reliable basis for a treatment decision, particularly in excluding the diagnosis of tuberculosis, due to its high sensitivity1. **Aims and Objectives:** To assess the importance of adenosine deaminase(ADA) level in the diagnosis of pleural effusion. To assess Adenosine Deaminase Activity (ADA) in tuberculosis pleural effusion and assess the sensitivity and specificity of ADA levels. **Materials and Methods:** This study was performed at the Department of Pulmonary Medicine at tertiary care centre. The study comprised of 75 patients of pleural effusion having Age > 14 years, Clinical and Radiological evidence of Pleural Effusions & Patients willing for ADA examination. Patients having Age > 65 years, minimal nontappable effusion, not giving consent for ADA examination patient were excluded from the study. Detailed history, thorough physical examination, radiological findings, haematological and biochemical findings were recorded in the proforma. Pleural aspiration was performed on all patients. Macroscopic findings, cytological, microbiological and biochemical analysis of pleural fluid were performed in all patients including ADA level. PCR for Mycobacterium tuberculosis was also assessed in pleural fluid. Pleural fluid Adenosine deaminase level was measured by Giusti and Galanti method. **Result:** In our study out of 45 patients with tuberculosis pleural effusion ADA was more than 40IU/L in 42 (93.33%) and less than40IU/L in 3 (6.66 %) . Our study showed a mean ADA of 107.7 IU/L Using a cut off of greater 40IU/L we got a sensitivity and specificity of 93.3% and 90% respectively and Positive predictive value 93.3% and Negative predictive value 90%. **Conclusion:** Pleural fluid ADA activity has been shown to be a valuable biochemical marker that has a high sensitivity and specificity for TB diagnosis.

Keywords: ADA, Pleural Effusion, Tuberculosis

1. Introduction

Pleural effusion is the abnormal accumulation of fluid in the pleural space.

A pleural effusion is always abnormal and indicates the presence of an underlying disease[2]. Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption. Normally, fluid enters the pleural space from the capillaries in the parietal pleura and is removed via the lymphatics situated in the parietal pleura. Fluid can also enter the pleural space from the interstitial spaces of the lung via the visceral pleura or from the peritoneal cavity via small holes in the diaphragm. The lymphatics have the capacity to absorb twenty times more fluid than is normally formed[4]. The first step in the evaluation of a pleural effusion is a detailed history and physical examination; the importance of the history and physical examination arises from the fact that a

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significant percentage of pleural effusions have no definitive diagnostic features on pleural fluid analysis or pleural biopsy.

Diagnosis of the cause of many pleural effusions is based on the clinical setting and exclusion of other alternative causes.

The next step is sampling of the pleural fluid and categorization as a transudate or exudate. Transudative pleural effusions result from systemic diseases like congestive heart failure, cirrhosis with ascites, and the nephrotic syndrome. Exudative pleural effusions result from local or systemic diseases that directly injure the pleural surface. The diagnostic focus for exudative effusions is to recognize the responsible intrapleural disease[2].

TB is the most common cause of pleural effusion worldwide (30-60%). In the United States, Tuberculous Pleural Effusion (TPE) accounts for 2 to 5% of all pleural effusions, approximately 1000 cases per year, and is the one of the most common extrapulmonary manifestation of tuberculosis[3].

It is important to consider the possibility of tuberculous pleuritis in all patients with an undiagnosed pleural effusion[5]. The stepwise diagnosis of TB pleural effusion is subsequently the same as for any other exudative pleural effusion. An initial diagnostic thoracocentesis is always indicated**.** Definitive diagnosis of Tubercular pleural effusion can be difficult to make because of low sensitivity and specificity of noninvasive diagnostic tools. Results of pleural fluid staining for Acid Fast Bacilli (AFB) are virtually always negative and pleural fluid cultures for mycobacterium are positive in **< 25%** of cases.

Pleural biopsy is the most useful investigation; it can establish the diagnosis in 95% of the cases in combination with the culture of biopsy specimen. But pleural biopsy cannot be done in every patient of pleural effusion because of lack of facilities and inadequate or minimal effusion. New tests which are diagnostic like ELISA detect mycobacterial antigens in tubercular exudates. And assay of specific antibody response to mycobacterial antigens; particularly in a patients with AIDS.

The diagnosis of pleural tuberculosis has been greatly improved by the use of biochemical markers, which are faster and can be more sensitive. Recently some workers have emphasised the importance of the estimation of ADA in pleural fluid. Adenosine deaminase; named ADA by Spencer et al. (1968); is a catalyst in purine metabolism. It's catalyses the irreversible deamination of adenosine to inosine. Its main physiological activities are related to lymphocytic proliferation and differentiation. Activity increases substantially during mitogenic and antigenic responses of lymphocyetes. As a marker of cellular immunity, its plasma activity is found to be elevated in the diseases in which there is cell mediated immune response.

The present study was aimed to evaluate the diagnostic efficacy of Adenosine Deaminase **(ADA)** levels in pleural effusions.

2. Aims and Objectives

- To assess the importance of adenosine deaminase (ADA) level in the diagnosis of pleural effusion.
- To assess Adenosine Deaminase Activity (ADA) in tuberculosis pleural effusion.
- To assess the sensitivity and specificity of ADA activity assay method.

3. Materials and Methods

This study was performed at the Department of Pulmonary Medicine at our centre.

The study comprised of 75 patients of pleural effusion having Age> 14 years, Clinical and Radiological evidence of Pleural Effusions & Patients willing for ADA examination. Excluding patients Age >65 years, minimal nontappable effusion, not giving consent for ADA examination patient.

Detailed history, thorough physical examination, radiological findings, haematological and biochemical findings were recorded in the proforma. Pleural aspiration was performed on all patients. Informed consent obtained from procedure obtained from all patients

Macroscopic findings, cytological, microbiological and biochemical analysis of pleural fluid were performed in all patients including ADA levels.

PCR for Mycobacterium tuberculosis was also assessed in pleural fluid.

Pleural fluid Adenosine deaminase level was measured by Giusti and Galanti method.

The ADA was measured by following formula

Calculations:

$$\text{Total ADA activity in U/L} = \frac{\text{Abs.T - Abs.SB}}{\text{Abs.S - Abs.B}} \times 50$$

4. Statistical Analysis

Continuous variables are presented as mean ±SD and frequency variables as percentages.

Chi-square test was performed for statistical significance. P value of <0.05 was considered for statistical significance.

5. Results

In this study, a total of 75 cases of pleural effusion were studied and the following observations were made of the 75 cases, the distributions of the various types of pleural effusion were as follows.

Table 1. Distribution of type of effusion

Sl. No	Type of Effusion	No. of Cases	Percentage
1.	Tuberculous	45	60
2.	Malignant	12	16
3.	Transudative	11	14
4.	Synpneumonic	07	10
	Total	**75**	**100%**

Among the 75 cases, 45 cases were tubercular effusion,12 cases malignant effusion, 11 transudative and 7 cases were synpneumonic effusion (Table 1)

Among the 75 cases of pleural effusion there were 54 males and 21 females.Thus out of total 75 cases, 72% were males and 28% were females. Pleural Effusion being more common in males than in female.

Pleural effusion was more common in age group of 26-55yrs TB pleural effusion was more common on left side & transudative on right Side. The most common presenting complaints were cough (73.33%) and chest pain (76.66%) followed by fever (70.00%), breathlessness (63.33), wt. Loss (60%) and loss of appetite (60%).

The average total leucocyte counts in Tubercular, Malignant, Transudative and Synpneumonic was pleural effusion 7792, 8026, 6770 & 12440 cells/mm^3 respectively.

The average ESR value in Tubercular, Malignant, Transudative, Synpneumonic pleural effusion 68, 45, 15.7&41.6 mm/hr respectively. ESR was significantly elevated in exudates.

Table 2. Showing the estimated mean ± SD of pleural fluid cell count and cell type.

Sl. No	Type of effusion	No of Cases	Cell count Cell/mm^3	Cell type predomnant	Malignant Cells
1.	Tubercular	45	1061±410	Lymphocytes	-
2.	Malignant	12	574±190	Lymphocytes	+ve in 8 cases
3.	Transudative	11	139±31	Monocytes and lymphocytes	
4.	Synpneumonic	07	1332±571	Polymorphocytes	-

Study shows cell type predominance in tubercular effusion, malignant effusion, transudative and synpnemonic effusion were lymphocytes, lymphocytes, lymphocytes and monocytes and polymorphocyte respectively (Table 2)

The mean pleural Fluid cell count in Tubercular, Malignant, Transudative and Synpneumonic are 1061 ±410,574 ±190,139 ±31 and 1332 ±571. (Table 2)

Table 3. Showing the estimated mean ± SD of pleural fluid glucose, protein& ADA

Sl. No	Type of effusion	No.of Cases	Pleural Fluid Sugar(mg)	Protein(gm)	ADA(IU/L)
1	Tubercular	45	53.16±10.92	4.83±0.75	122.02±57.45
2	Malignant	12	64.37±8.86	3.56±0.47	31.75±5.51
3	Transudative	11	103.85±26.14	2.42±0.63	27.14±5.78
4	Synpneumonic	07	39.6±9.39	4.08±0.33	28.0±9.46

Study shows the mean pleural Fluid sugar in Tubercular, Malignant, Transudative and Synpneumonic are 53.16±10.92, 64.37±8.86, 103.85±26.14 and 39.6±9.39. sugars were found to be low in the Synpneumonic pleural effusions.The mean pleural fluid protein in tubercular, malignant, transudative and synpneumonic are 4.83+/-0.75;3.56+/-0.47;2.42+/-0.63 and 4.08+/-0.33.protein was found significantly high in TB pleural effusion (Table 3)

The Mean ADA (IU/L) in pleural Fluid in Tubercular, Malignant, Transudative and Synpneumonic 122.02±57.45, 31.75±5.51, 27.14±5.78 and 28.0±9.46. The mean ADA of TB effusion is 107.7 IU/L and of Non TB effusion is 30.7IU/L.

6. Discussion

The most frequent cause of pleural effusion in India is tuberculosis. But at times pleural effusion can be a presentation of various other diseases. Even after extensive investigations some pleural effusions remain undiagnosed. Routine investigations of pleural fluid can sometimes helps in etiological diagnosis, but not in all cases. Exudative Lymphocytic pleural effusions are commonly encountered in clinical practice but they often constitute difficult diagnostic problems.

Adenosine Deaminase is an enzyme in the purine salvage pathway required for converting Adenosine to Inosine. Its levels are ten times higher in lymphocytes than in erythrocytes and particularly in T-lymphocytes.

TB Pleural effusion is the manifestation of delayed hypersensitivity to *Mycobacterium tuberculosis* antigen and is characterized by the presence of activated T Lymphocytes and macrophages in the pleural space.

Elevated levels of ADA in TB Pleural effusion have been noted by several authors. These observations were reproduced and further confirmed in our study. In this

prospective study of 75 patients with pleural effusion, the mean age was 36 years and two thirds were men.

In comparison, the sex distributions in some of the previous studies are: Leesly J. Burgess[6] - 58% males and 42 % females, Luis Valdes[7]- 56.6% males and 43.3% females.

The three most common causes of exudative effusion in this study were tuberculosis, malignancy and Synpneumonic effusions.

The patients with TB were younger than the patients with malignancy.

In this study the mean age of TB Pleural effusion was 31 years, consistent with Luis Valdes et al (34 years) and S.K.Sharma et al (33 years)[8].

In one recent series from Qatar, Ibrahim WH et al[9] reported the mean age of 100 patients with tuberculous pleuritis was 31.5 years. Denise Duprat Neves et al[10], the mean age in patients with TB (mean = 33.76; SD = 13.96 years old) was significant lower ($p < 0.0001$) than in NTB group (mean = 49.29; SD = 18.01 years old).

The commonest exudative effusion in this study was tuberculosis (60%) followed by malignant effusion (16%) and synpneumonic effusion (10%).

In India tubercular effusion is the commonest cause of all exudative effusions. This is similar to the observation in another study from India by Maldhure et al where they showed that the tubercular effusions constitute 66% of the effusions, malignancy 15%, and parapneumonic effusion 4.8%. This observation is different from that of the West where the incidence of parapneumonic effusion and malignant effusion are much higher compared to that of tubercular effusion. This is consistent with the fact that India has a high prevalence of tuberculosis in the general population.

The most common symptom encountered by our TB patients were fever (76%) followed by cough (73%) and chest pain (70%). These findings are compatible with the studies done earlier by Moudgil et al[11].

The symptoms most commonly reported in published series by Morehead RS et al[12] are: cough (71-94%), fever (71-100%), chest pain (78-82%) and dyspnea.

Patients with malignant effusion had dyspnoea as a common symptom (51%) similar to that seen in a study by Chernov B et al. Patients with synpneumonic effusion had clinical symptoms suggestive of pneumonic illness.

In our study we demonstrated that massive effusion was most commonly seen in malignant effusion group (50 %) similar to that observed, by Maher et al (55%)[13].

Large effusions were less commonly seen in the other observed etiologies.

Although the majority of effusions were straw colored, hemorrhagic effusions were encountered predominantly in malignant effusions.

Ten percent of tuberculous effusions in our study had a total count greater than 10,000/mm^3 similar to Light's[14] observation. Ninety percent of TB effusions and most of malignant effusions had lymphocyte predominance.

Our result was similar to the study done by Valdes L et al where they have encountered neutrophil predominant tuberculous effusion in only 6.7% of patients and only one malignant effusion had neutrophil predominant effusion (3%).

The average Hb% in Tubercular effusion, Malignant, Transudative and Synpneumonic pleural effusion was 9.616, 8.412, 8.6 and 11.76 % respectively.

The average total counts in Tubercular, Malignant, Transudative and Synpneumonic was pleural effusion 7792, 8026, 6770 and 12440 cells/mm^3 respectively.The average ESR value in Tubercular, Malignant, Transudative, Synpneumonic pleural effusion 68, 45, 15.7 and 41.6 mm/hr respectively. ESR was significantly elevated in exudates.

Low pleural fluid glucose was seen predominantly in patients with Synpneumonic effusion.

The majority of pleural fluid glucose levels were between 40-100mg/dl in tubercular effusions, consistent with the earlier observation by Light. Only 3 % of tuberculous effusions had sugars less than 40mg%.

According to the literature pleural fluid adenosine deaminase (ADA) has got a good discriminative value in differentiating tuberculous effusions from malignant effusion.

Although a pleural fluid ADA above 70IU/L is diagnostic of tuberculosis it has to be considered if the pleural fluid ADA is between 40 IU/L and 70 IU/L. The variations may be due to-

- Different optimal conditions used to carry out the experiment e.g. temperature in the water bath, spectrophotometer wavelength, light path distance etc.
- Laboratory set up and technical variation; errors.
- Concentration of the reagent used.
- Storage of reagent and old reagents.
- Solutions not prepared in ammonia free distilled water.

An ADA level less than 40IU/L rules out pleural tuberculosis.

In our study out of 45 patients with tuberculosis pleural effusion 42 (93.33%) of them had a level more than 40IU/L but 3 (6.66 %) showed a level of less than 40IU/L. Studies done in the West demonstrate pleural fluid ADA more than 70 IU/L (Valdes et al and Burgess et al), our study showed a mean of 107.7 IU/L. The mean ADA were high in the 2 Indian studies done by Rajendra Prasad et al[15], and Gilhotra et al[16] with the mean ADA level ranging between76.8 IU (+/_ 23.8) to 95.8 (+/_57.5)

We determined the sensitivity and specificity of ADA in patients of tuberculosis. Using a cut off of greater 40IU/L we got a sensitivity and specificity of 93.3% and

90% respectively and Positive predictive value 93.3% and Negative predictive value 90%.

This is more consistent with the observation made by Valdes et al[7]. Spain 47 IU/L cut off value sensitivity 100%, specificity 95%, Positive predictive value 85%, Negative predictive value 100% with mean ADA 107.5

All our malignant effusions had pleural fluid ADA less than 40 IU/L with a mean of 32.92.

All the other Indian studies also showed a similar finding, where the average pleural fluid ADA among malignant effusions were 7 to 18 IU/L.

Table 4. Comparison of different studies of pleural fluid ADA

Authors	TB pl. Effusion		Non TB pleural Effusion	
	N	Pleural fluid ADA(IU/L) M ± SD	N	Pleural fluid ADA(IU/L) M ± SD
Sharma et al[4]	47	95.8±57.5	27	30.7±27.2
M.-L. Chen et al	63	78.17±24.76	147	27.64±26.10
Present Study	45	107.7±31.95	30	30.78±8.59

Here there is comparison of sharma et al, M-L.Chen et al and our study of ADA in tuberculous effusion and nontuberculous effusion (Table 4)

In this study there was a statistical significant association (p value <0.05) of ADA levels in differentiating TB pleural effusion from Non TB pleural effusion. (Table 4)

ADA estimation is a quick and easy method of diagnosing tubercular pleural effusion. Considering the wide variations in the values mentioned for the ADA activity as regards to the diagnosis of tubercular pleural effusion in various studies; it would be advisable that each laboratory or institute sets up its own standard for ADA values for the diagnosis of tubercular pleural effusion.

7. Summary and Conclusion

- Pleural effusion is a commonly encountered in medical practice and in our country, the commonest cause is tuberculosis.
- The initial step in evaluating case of pleural effusion is to establish the cause of pleural effusion which is done by a detailed history, clinical examination and investigations like a chest radiology and pleural fluid analysis. Pleural fluid ADA activity has been shown to be a valuable biochemical marker that has a high sensitivity and specificity for TB diagnosis.
- In our study Pleural effusion was more common in age group of 26-55 years.
- Mean age group of TB pleural effusion is 31 years and common in men.
- The patients with TB were younger than the patients with malignancy.
- All patients with TB Pleural effusion had elevated ADA levels in Pleural fluid.
- In our study out of 45 patients with tuberculosis pleural effusion ADA was more than 40 IU/L in 42 (93.33%) and less than40IU/L in 3 (6.66 %).
- Our study showed a mean ADA of 107.7 IU/L
- In this study there was a statistical significant association (p value < 0.05) of ADA levels in differentiating TB pleural effusion from Non TB pleural effusion.
- Using a cut off of greater 40 IU/L we got a sensitivity and specificity of 93.3% and 90% respectively and Positive predictive value 93.3% and Negative predictive value 90%.
- Thus pleural fluid ADA estimation seems to have the potential for being one of reliable test for the diagnosis of TB pleural Effusion which is adequately sensitive and specific and at the same time or rapid, inexpensive and easy to perform.

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