

Adenocine deaminase activity in pleural effusion due to various etiologies and its utility as a dignostic tool in tuberculous pleural effusion

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Abstract

Introduction: To Document ADA activity in Pleural Effusion of Varied Etiologies, This Study was conducted. The Laboratory Technique of ADA level Estimation is a simple Procedure. The Pleural Effusions of Various etiologies are broadly grouped as Tuberculous and Nontuberculous. The Diffrence in ADA levels in these Groups is statistically analysed. **Objective:** To Compare the ADA activity in Pleural Effusion due to Varied Etiologies and to see its utility in diagnosing TB pleural Effusion. **Methodology:** This prospective study was undertaken over a period of Two years consisting of 122 adult (age>13yrs) patients of Pleural Effusion with varied etiology. These Patients were divided into three groups as- Group A- Tuberculous Pleural Effusion and Nontuberculous Groups, Group B- Malingnant Pleural Effusion, and Group C-Transudative Pleural Effusion, depending upon Pleural fluid analysis for Biochemistry, Cytology and Microbiology with other relevant investigations like FNAC of lymphnode or lung lesion as required. ADA levels of pleural fluid in all these patients were determined by Gusti and Galantis Calorimetric method. The whole data is collected, tabulated and statistically analysed. **Results:** In this study, it was observed that, by using ADA cut off value of 60 IU/LIT, Sensitivity, Specificity, Positive predictive value, and Negative predictive value of the test were 77.89%,92.59%,97.39%,54.34% respectively. LR + was found to be 10.52 and LR – was found to be 0.24.

Conclusion: The ADA Level Determination, Probably a good Diagnostic Test with High Specificity for Tuberculous Pleural Effusion.

Keywords: Adenosine Deaminase (ADA), Pleural Effusion

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INTRODUCTION

Pleural effusion itself is not a disease entity; it must be regarded as a manifestation of various diseases. Accumulation of excess amount of fluid in the Pleural space is a serious manifestation of thoracic diseases, usually Pulmonary or Cardiac and occasionally as a first evidence of an important Extra-Thoracic or Systemic

Disease. To document ADA activity in Pleural effusions secondary to Tuberculous and Nontuberculous causes and to compare, this study was conducted. The laboratory technique of ADA level estimation is a simple Procedure. If this study results reveals statistically significant levels of ADA, this test will be recommended for diagnosis of tuberculous pleural effusion.

MATERIAL AND METHODS

Study Population

One hundred and Twenty two adult patients from DR. SCGMC and Hospital, Nanded were enrolled in to study over a period of two years.

Inclusion Criteria

We have included the patients whose x ray Chest suggestive of pleural effusion and which is undiagnosed yet, with 1) Age>13yrs 2) who are ready to participate in the study.

Exclusion Criteria

We have excluded the patients with: 1) age < 13 yrs 2) Diagnosed Pleural Effusion already on treatment. 3) Who are not willing to participate in the study.

Methodology

This Prospective Study was undertaken over a period of Two years consisting of 122 patients of pleural effusion with varied etiology. These Patients were divided into three groups as- Group A- Tuberculous Pleural Effusion, Group B- Malignant Pleural Effusion, and Group C- Transudative Pleural Effusion, depending upon Pleural fluid analysis for Biochemistry, Cytology and Microbiology with other Relevant Investigations like FNAC of lymphnode or lung lesion as required. ADA levels of pleural fluid in all these Patients were determined by Gusti and Galantis Calorimetric method. The Mean value of ADA level in Group A was compared with the mean value of ADA level in Both the Non Tuberculous Groups i.e. Group B and Group C by applying statistical test “Testing of hypothesis at 95% level of significance when mean and standard deviation of

two samples is given.” Sensitivity, Specificity, and Likelihood Ratio of ADA for prediction of tuberculosis were determined using ADA cut off value of 60 IU/ LIT.

Statistical Analysis

Upon collection of the data, By using ADA cut off value of 60 IU/Lit., Sensitivity, Specificity Positive predictive Value, Negative predictive value, false positive, false negative rate with Likelihood Ratio +VE and Likelihood Ratio -VE are Calculated. For comparision of ADA activity in Tuberculous (Group A) and Nontuberculous Groups (B and C), the statistical test applied is “Testing of Hypothesis at 95% level of Significance, when mean and standard deviation of two samples is given.”

RESULTS

This study included 122 Patients of Pleural Effusion. Patients were divided into according etiology of Pleural Effusion as follows:

Table 1: Study population – Etiological Distribution

Sr. No.	Etiological Type	No. of patients	Percentage (%)
1	Tuberculous (Group A)	95	77.87%
2	Malignant (Group B)	15	12.30%
3	Transudative (Group C)	12	9.83%
TOTAL		122	100%

Table 2: Study population – Sex distribution

Group A (Total=95)				Group B (Total=15)				Group C (Total=12)			
Male	%	Female	%	Male	%	Female	%	Male	%	Female	%
75	78.95	20	21.05	12	80	03	20	08	66.67	04	33.33

From above table it was observed that 75 patients were Male (78.95%) and 20 Patients were female (21.05%) in Group A. In Group B, 12 Patients were male (80%) and 3

patients were females (20%), while in Group C, 8were male patients (66.67%) and 4 patients were female (33.33%).

Table 3: Age Group Distribution

Sr. No.	Age	Group A		Group B		Group C	
		No.	%	No.	%	No.	%
1	<12	00	00	00	00	00	00
2	13-24	21	22.10	00	00	02	16.67
3	25-34	26	27.37	00	00	03	25
4	35-44	20	21.05	03	20	02	16.67
5	45-54	12	12.63	00	00	03	25
6	55-64	09	9.47	06	40	02	16.67
7	>65	07	7.37	06	40	00	00
TOTAL		95	100%	15	100%	12	100%

From above table, it is observed that the most commonly affected age group in Group – A was 25- 44 (48.42), in Group –B out of 15 patients, 12 were above the age of 55

years (80%), while there was varied age group distribution in Group – C.

Table 4: Pleural Fluid ADA Activity in Group A

Sr. No.	ADA Range(IU/L)	Pleural ADA activity		Mean ADA Level
		NO.	%	
1	0-60	29	30.52	94.82 +_46.63IU/LIT
2	61-120	49	51.57	
3	121-180	10	10.52	
4	181-240	05	5.26	
5	>241	02	2.10	
TOTAL		95	100%	

The Above table indicates that, in Group – A i.e. Tuberculous Pleural Effusion, in maximum number of patients, ADA activity was in the range of 61-120 IU/LIT i.e. in 49 patients (51.57%). The next common range was

0-60, 29 patients. (30.52%) The highest Value of ADA level in Group A was 252.27IU/LIT and the lowest value was 22.61 IU/LIT. The mean ADA level in the Group A was 94.82 +_46.63IU/LIT

Table 5: Pleural Fluid ADA Activity in Group B

NO.	ADA Range(IU/L)	Pleural ADA activity		Mean ADA Level
		NO.	%	
1	0-20	03	20	41.60 +_24.11 IU/LIT
2	21-40	08	53.33	
3	41-60	02	13.33	
4	61-80	01	6.67	
5	>81	01	6.67	
TOTAL		15	100%	

In Group B patients Fluid ADA Activity was maximum in the range of 0-40IU/LIT, The mean ADA activity in

this group was 41.60 +_24.11 IU/LIT

Table 6: Pleural Fluid ADA Activity in Group C

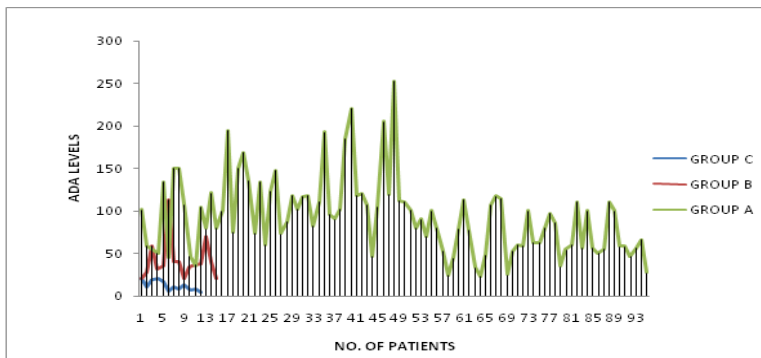
Sr. NO.	ADA Range(IU/L)	Pleural ADA activity		Mean ADA Level
		NO.	%	
1	0-5	00	00	11.79 +_5.60 IU/LIT
2	6-10	06	50	
3	11-20	05	41.67	
4	>20	01	8.33	
TOTAL		12	100%	

In Group C, patients Fluid ADA Activity was maximum in the range of 6-20/LIT, The mean ADA activity in this

group was 11.79 +_5.60 IU/LIT.

Table 7: Comparative Study of Pleural Fluid Mean ADA In 3 Groups

Sr. No.	Groups	No.	Mean ADA Activity
1	A= Tuberculous	95	94.82 +_46.63IU/LIT
2	B= Malignant	15	41.60 +_24.11 IU/LIT
3	C= Transudative	12	11.79 +_5.60 IU/LIT



Graph 1: Comparison OF ADA Levels In Three Groups

Table 8 a: Statistical Evaluation of Test Group A and B

	Mean ADA	Statistical Analysis
Group A	94.82 + _{46.63} IU/LIT	Z tab < Z cal i.e. 1.96 < 4.40
Group B	41.60 + _{24.11} IU/LIT	

Table 8 b: Statistical Evaluation of Test Group A and B

	Mean ADA	Statistical Analysis
Group A	94.82 + _{46.63} IU/LIT	Z tab < Z cal i.e. 1.96 < 6.14
Group C	11.79 + _{5.60} IU/LIT	

The statistical test applied is ‘Testing of Hypothesis at 95% level of Significance’ when mean and standard deviation of two samples is given. From above table a and b, there was a statistically significant increase in the mean value of ADA of Tuberculous group (94.82

+_{46.63} IU/LIT) than malignant group (41.60 +_{24.11} IU/LIT) and Transudative group (11.79 +_{5.60} IU/LIT) since tabulated Z value (1.96) is less than calculated Z value in both, Table a i.e. 4.40 and Table b i.e. 6.14.

Table 9: Calculating Sensitivity, Specificity, Likelihood Ratio*(LR) For prediction of Tuberculosis

	Tuberculous	Nontuberculous (Malignant + Transudative)	Total
ADA >60	74	02	76
ADA <60	21	25	46
TOTAL	95	27	122

Table 10

ADA cut off value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	False +ve	False -ve	LR+	LR-
60	77.89%	92.59%	97.37%	54.34%	7.40%	22.10%	10.52	0.24

From above table it is observed that by using ADA cut off value of 60 IU/LIT for prediction of Tuberculous pleural Effusion Sensitivity, Specificity, Positive Predictive value, Negative predictive value of the tests will be 77.89, 92.59, 97.37, 54.34 respectively. This test has 7.40 % false positive and 22.10% false negative value. The LR Positive and LR Negative of the tests are found to be 10.52 and 0, 24 respectively.

DISCUSSION

In Recent years the evaluation of ADA activity in pleural fluid is proven to be sensitive, specific and thus useful for the diagnosis of tuberculous pleural effusion. The ADA catalyzes the conversion of adenosine and

deoxyadenosine to inosine and deoxyinosine respectively. The ADA is detected in lymphocytes primarily hence ADA activity in the pleural fluid reflects the cellularity in the pleural compartment, particularly activated T lymphocytes. Tuberculosis is a common problem in developing countries like India. It can affect any system but the commonest is the respiratory. Tubercular etiology is a common cause of Pleural effusion. Tuberculosis has been diagnosed traditionally by assessing the presence of bacilli in the culture or AFB in clinical specimen. However sensitivity of AFB staining is low as the disease is paucibacillary. The ‘Gold Standard’ culture procedure takes up to 3 weeks. The present study is intended to know the utility as a diagnostic parameter of estimation of value of ADA in

patients of pleural effusion of varied etiology. In Present study one hundred twenty two patients of pleural effusion of various etiologies like tubercular, malignant, and transudative were studied with reference to ADA activity in pleural fluid. The patients were divided into 3 Groups according to etiology

Group A: Tuberculous Pleural Effusion.

Group B: Malignant Pleural Effusion.

Group C: Transudative Pleural Effusion.

Sex Incidence

In present study there were 95 males and 27 females with male to female ratio of 1:0.28. If Group A and B are considered Male predominance was there, which coincides with the results from the studies of Berger *et al* (1973)¹, Levine *et al* (1968)², Sharma S. (1988)³ and Reechaipichitkul *et al* (2001)⁴ while in group C male to female ratio was 1:0.5. It means that ratio has changed depending on etiology, but was not found to have any relation with the diagnosis.

Significance of Age

Considering overall study of 122 Patients, ages of the patients ranged from 14 to 75 years. In Group A i.e. Tuberculosis Pleural effusion majority of patients i.e. 70.52% were below 44 years of age, with mean age of 36.53+_{14.36} years. Arif Rahim *et al* (2002)⁵ found mean age of 28.70+_{9.73} in patients of Tuberculous Pleural effusion. The age group commonly affected in malignant effusion was patients with age >55 years (>80%) with mean age 59+_{10.86} years. Mathur K S *et al* (1961)⁶ found 60% patients above the age of 40 years. Hirsh (1979)⁷ found mean age of 53 years in malignant pleural effusion. In Group C i.e. Transudative Pleural effusion which was common in 25-35 years and 45-54 years of age group (25%) each which correlates with the study of Sharma *et al* (2001)⁸ who found varied age group distribution between 17-75 years for Transudative effusion.

ADA activity in Three Groups:

Our observations were-

1. The mean ADA levels in
 - i. Tuberculous Pleural effusion was 94.82 +_{46.63} IU/LIT
 - ii. Malignant Pleural effusion was 41.60 +_{24.11} IU/LIT
 - iii. Transudative Pleural Effusion was 11.79 +_{5.60} IU/LIT
2. ADA activity is found to be significantly higher in Tuberculous effusion when compared to malignant and transudative effusions.
3. Sensitivity and Specificity of the test is found to be 77.89% and 92.59% respectively.

4. In Nontuberculous effusions ADA activity is low. Thus a value in the low range is of no diagnostic help except in excluding tuberculosis.

Sharma S.K. *et al* (2001)⁸ prospectively studied usefulness of ADA estimation in tuberculous pleural effusion (n=75) and conclude using cut off value of 100 IU/LIT. It is possible to avoid pleural biopsy. Arif Rahim *et al* (2002)⁵ also found high ADA level (40.2+_{10.82} IU/LIT) in Tuberculous Pleural Effusion (n=60) than that of non tuberculous group (n=20) (24+₁₁ IU/LIT) while studying 80 patients of pleural effusion. Meena Varma *et al* (2004)⁹ found a level of tuberculous fluid ADA in the range of 64.48 to 102.44 while studying in 100 patients of pleural effusion with different etiology. Ernam D. *et al* (2005)¹⁰ studied 226 patients of pleural effusion of different etiology and showed high level of pleural ADA alone can be indicative of tuberculous pleural effusion.

Comparison of mean ADA levels in pleural fluids of tubercular, malignant and transudative effusion as reported by others studies

Table 11: Mean ADA levels in IU/LIT

Study	Tubercular	Malignant	Transudative
Sinha P.K.11 <i>et al</i>	76.80+ _{23.80}	14.50+ _{8.90}	5.00+ _{2.00}
Inama Ocana12 <i>et al</i>	92.43+ _{29.43}	13.43+ _{10.00}	2.29+ _{3.4}
Piras M.A.13 <i>et al</i>	83.04+ _{25.51}	15.54+ _{6.74}	17.26+ _{0.75}
F.J. Maritz14 <i>et al</i>	92.11+ _{37.05}	22.23+ _{13.15}	----
R.K.Narang15 <i>et al</i>	97.70+ _{16.00}	19.40+ _{4.80}	8.70+ _{0.40}
Subhakar16 <i>et al</i>	66.41+ _{29.31}	17.32+ _{2.64}	9.50+ _{0.99}
Chopra17 <i>et al</i>	99.56+ _{9.78}	20.28+ _{3.04}	12.25+ _{1.41}
Present study	94.82+ _{46.63}	41.60+ _{24.11}	11.79+ _{5.60}

Sensitivity, Specificity, and Likelihood Ratio (LR) of ADA level estimation Test:

LR(+)

Ratio of sensitivity of test to false positive error rate of the test. Higher the ratio better the test. For the test to be good one the ratio should be much larger than one.

LR(-)

Ratio of false negative error of test to specificity of the test. Smaller the ratio (i.e. closer to zero) better the test. In summary, if LR + of a test is large and LR- is small. It is probably good diagnostic test¹⁸. In Present study, Likelihood Ratio Positive and Likelihood Ratio Negative of the tests are found to be 10.52 and 0.24. Sensitivity, Specificity, Positive Predictive value, Negative predictive value of the tests will be 77.89, 92.59, 97.37, 54.34 respectively. Our calculated values correlates with the study done by Reechaipichitkul *et al* (2001)⁴, who yielded sensitivity, specificity, positive predictive value and negative predictive value of 80%, 80.5%, 71.4%, and

86.8% respectively with ADA cut off value of 48 IU/LIT. The tests Likelihood Ratio was 5:2. To Conclude, Adenosine Deaminase (ADA) is an enzyme of Purine Catabolism, participates in the degradation of Purines by converting adenosine to inosine and deoxyadenosine to deoxyinosine. ADA is present in various body fluids. It is essential for the differentiation and proliferation of lymphoid cells Particularly T-cell [Piras *et al*¹³]. ADA is a predominant T-Lymphocyte enzyme and its plasma activity is high in disease where cell mediated immunity is stimulated and agress well with the negative response observed in combined immuno deficiency. Tom Petterson *et al*¹⁹, suggested that higher ADA activity in pleural fluid was probably due to ADA being synthesized by the cells in the pleural cavity in tuberculosis, a reflection of local activation of selectively sequestred T-lymphocytes a local cellular immune response. Although the origin of pleural fluid ADA and the role of lymphocytes in pleural effusions remain to be determined, it seems reasonable to regard the high ADA activity in tuberculous pleural effusion as a reflection of local activation of T-Lymphocytes i.e. Local cellular immune response. However, further studies are needed to support our conclusions in this regard.

CONCLUSION

1. Mean Values of ADA in Tuberculous Pleural Fluid is 3-4 times raised significantly as compared to Malignant and Transudative Pleural Effusions.
2. The increase in ADA activity in Tuberculous Pleural effusion cases was statistically significant as compared to Nontuberculous Pleural effusions.
3. Sensitivity and Specificity of the test were found to be 77.89% and 92.59% respectively for Diagnosis of Tuberculous Pleural Effusion.
4. The Likelihood Ratio (+) and Likelihood Ratio(-) of the tests were 10.52 and 0.24 respectively. This makes the ADA level determination, probably a good diagnostic test with high specificity.

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