

STUDY OF ADENOSINE DEAMINASE AND LYMPHOCYTE/NEUTROPHIL RATIO IN COMBINATION AS DIAGNOSTIC TOOL FOR TUBERCULAR PLEURAL EFFUSION

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ABSTRACT: Adenosine deaminase, considered one of the key enzyme of purine metabolism, has been used in work up of lymphocytic pleural effusion. Low level of ADA <40IU/L essentially excludes tuberculosis from consideration as differential diagnosis of pleural effusion cases. ADA >50IU/L specially when combined with Lymphocytic/neutrophil ratio >0.75 in pleural fluid is useful test in the diagnosis of tubercular pleurisy.

AIM AND OBJECTIVE: To suggest a better diagnostic tool in the diagnosis of pleural effusion of tubercular origin by estimating the activity of ADA along with L/N ratio in pleural effusion.

METHOD: Biochemical, cytological and microbiology studies were done by obtaining pleural fluid by thoracocentesis in 100 patients after excluding pleural effusion cases of malignancy, transudative effusion.

RESULT: 84 cases were tubercular and had high level of ADA in comparison to rest of 16 non-tubercular cases. At level of 50 IU/L of ADA activity test had sensitivity of 97.6%, specificity 87.5%, positive predictive value 97.6%, negative predictive value 87.5% which increased to 100% and 92.8%, 98.6%, and 100% respectively in combination with test of Lymphocytic/Neutrophilic ratio >0.75.

CONCLUSION: ADA level with L/N ratio can be important investigation in diagnosis of tubercular pleural effusion cases.

KEYWORDS: Tuberculosis, Pleural Effusion, Adenosine Deaminase.

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INTRODUCTION: Tuberculosis is one of the oldest and commonest infectious disease known to mankind. In India and third world countries, it has always been a major health problems. In spite of presence of effective drugs, epidemiological data show worldwide rise in incidence since emergence of acquired immunodeficiency syndrome (AIDS). Being commonest pulmonary tuberculosis is often associated with effusion, which is second most common extra pulmonary clinical manifestation it. In India most common cause of pleural effusion is tuberculosis. Delayed in diagnosis or untreated cases may develop in to active tuberculosis and poor prognosis thus it is of extreme importance to diagnose and give treatment to the cases as early as possible.

Tuberculous pleurisy is thought to be the result of a delayed hypersensitivity reaction in response to the presence of mycobacterial antigen in response of mycobacterial antigen in pleural space. This immunologic reaction causes the stimulation and differentiation of lymphocyte which release lymphokines which in turn activate macrophages for enhanced bactericidal effect.¹

In addition to routine investigations, chest X-ray thoracocentesis is required to ascertain the nature of effusion and to differentiate it from manifestation of other etiologies.

Malignancies, infectious diseases, pulmonary embolism collagen vascular diseases, sarcoidosis, uremia are few of other causes of pleural effusion. Definitive diagnosis of tubercular pleural effusion is done by tubercular bacilli or pleural granulomas demonstration in effusion fluid or pleural biopsy specimen or sputum.

Adenosine deaminase is a useful chemical biomarker as screening test specially in endemic areas, origin of which is unknown and tuberculosis is virtually excluded if levels of ADA are very low. The determination of ADA activity was first proposed as serological marker for lung cancer in 1970.² Later, Piras et al in 1978³ reported the usefulness of ADA in diagnosing tubercular pleurisy. The presence of ADA in pleural fluid reflects the cellular immune response in pleural cavity and in particularly the activation of T lymphocyte. Several studies have suggested that an elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of 90-100% and a specificity of 89-100% when Giusti method is used.^{4,5,6,7} The reported cut off value for ADA (Total) varies from 47 to 60 IU/L.^{8,9,10}

Several studies have shown that ADA activity, especially when combined with differential leucocyte count and lymphocyte/ neutrophil ratio in pleural fluid remain a useful test in the diagnosis of tuberculous pleuritis. When the lymphocyte to neutrophils ratio (L/N ratio) >0.75 in pleural fluid was considered together with ADA activity >50IU/L, the results improved considerably for the diagnosis of tubercular pleuritis.¹¹

This study made an attempt to investigate the role of Adenosine deaminase activity and lymphocytic/neutrophil ratio in combination to diagnosis of tubercular pleural effusion.

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MATERIALS AND METHODS: The present hospital based case control study was undertaken in Department of Biochemistry Katihar Medical College and Hospital Katihar Bihar during the period from December 2007 to April 2009.

During this period of time 100 subjects were taken from admitted patients suffering from pleural effusion and selected by simple random method. Patients were informed about study and written consent was taken before any investigation. All exudative pleural effusion cases were included and patients with transudative pleural effusion e.g. congestive heart failure, rheumatoid arthritis were excluded from this study. Along with thorough clinical history and examination cases were investigated for following:

Haematological Examination: Haemoglobin, Total Leucocyte count, Differential leucocyte count, Erythrocyte sediment rate

- Chest X ray, P. A. View.
- Mantoux test.
- Sputum for AFB.

Pleural Fluid Examination: Differential count and Lymphocyte/Neutrophil ratio by microscope, LDH, Activity of Adenosine deaminase by using commercial kit, Z N stain for AFB, protein concentration.

Diagnostic thoracentesis was performed to obtain pleural fluid. Chest radiograph was obtained and localization of effusion was done. Physical examination such as diminished breath sound at the base of affected lung and decreased percussion note were performed to define the place. Patient were asked to take upright and sitting position with arms up and forward. Puncture site was marked with pen in mid-scapular line two rib interspaces down from upper end of effusion. Skin was cleaned with antiseptic solution over an area of 4 inches in all direction. Skin was anaesthetized by injecting 2% lidocaine site was punctured with 18 guaze needle attached to 50–60ml syringe containing heparin 1ml, advanced until feeling a slight give was obtained.

Estimation of Adenosine Deaminase activity in pleural fluid was done by method described by Guisti and Galanti. Only fresh collection were used. Berthelot reaction is the basis of calorimetric method in which ammonia and inosine produced due to ADA reaction on adenosine. Blue indophenol complex is produced due to reaction between ammonia, phenol and hypochlorite in alkaline medium.

Light's criteria.¹² (Pleural fluid protein/serum protein >0.5; pleural effusion fluid LDH/serum LDH >0.6) was used to exclude transudative pleural effusion. Cases of pleural effusion associated with malignancy/presence of cytological or histological evidence of malignancy were reviewed and included.

Diagnosis of tubercular pleural effusion was made on the basis of first or more than one of following criteria in addition to already proved diagnosed cases of TB:

1. Identification of Mycobacterium tuberculosis in pleural fluid or in sputum.
2. Clinical and radiological evidence of TB.
3. Clinically presenting with signs and symptoms consistent with TB exclusion of other clinical entity.
4. Definite clinical and radiological improvement in 6 to 8 weeks after administration of antitubercular treatment.

Method Analysis: Data were presented as frequency, percentage and mean±2SD. Student t test was applied to determine the significance of biochemical parameters between two groups. Pearson coefficient correlation were calculated for relationship between measured parameters, p value of <0.05 considered as significant. Data was analysed using statistical package program SPSS 15.0.

RESULTS AND DISCUSSION: In one and half years of the study period 100 cases of pleural effusion were studied and included by using simple random method. Out of 100 cases 84 were confirmed cases of tubercular pleural effusion and rest 16 cases were non-tubercular pleural effusion cases.

Among 84 tubercular pleural effusion patients 49(58.3%) were male and 35(41.6%) females. Subjects were classified in age groups <5, 5-15, >15 and mean age was 26.07 with SD±18.93277 (Range 4 to 67 years) (Table 1).

35.71% cases among tubercular pleural effusion (n=84) were positive for sputum smear (Table 2). All cases of non-tubercular pleural effusion were negative for sputum smear. Table 2 shows sensitivity and specificity of sputum smear for tubercular pleural effusion.

With Cut off level of ADA >50 IU/L, out of 84 cases of tubercular effusion 82 and 2 cases of non-tubercular cases had level of ADA >50 IU/L (Table 3). This cut off level of 50 IU/L had sensitivity of 97.6%, specificity of 87.5%, positive predictive value 97.6% and negative predictive value 87.5%. Observation made in this study were similar to previous studies.^{12,13,14}

Table 4 shows that 81 cases of tubercular pleural effusion and 2 cases of non-tubercular had Lymphocytic/Neutrophil ratio >0.75 out of total 84 cases and 16 cases respectively. Sensitivity was 96.4% and Specificity 87.7%. Positive Predictive value and Negative Predictive Value was 97.5% and 82.3% respectively.

Various studies.¹¹ had shown that combining ADA activity and Lymphocytic/Neutrophil ratio for diagnosis of tubercular pleural effusion increases sensitivity and specificity of these tests. In our study combination of these two had increased sensitivity (100%), specificity (92.8%), positive predictive value (98.6%) and negative predictive value (100%).

Table 6 shows comparison of mean between L/N ratio and ADA activity in pleural fluid in patients suffering from tuberculosis. Statistical analysis done by paired sample t test which concludes that two parameter positively correlate significantly (p=0.000).

The mean value of ADA activity in tubercular patients remains within the cut-off value and mean value of L/N ration in these patients are >0.75.

CONCLUSION: Tubercular pleural effusion traditionally diagnosed by identification of Mycobacterium tuberculosis in pleural fluid or culture of biopsy specimen. Clinical diagnosis are needed to be made before availability of lab results and this presents difficulty in practice. ADA level activity estimation is a simple, inexpensive, and in conjugation with L/N ration highly specific and sensitive test. Estimation of ADA along with lymphocytic/neutrophil ratio will provide less time consuming diagnosis of tubercular pleuritis and should be routinely included in pleural fluid examination.

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Ethical Clearance: The study was approved by ethical committee of Katihar Medical College, Katihar, Bihar.

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Age Group (years)	Male	Female	Total (% of cases n=84)
<5	0	3	3(3.5)
5-15	20	14	34(40.4)
>15	29	18	47(55.9)
Total	49	35	84

Table 1: Distribution of Cases of Tuberculosis according to Age and Gender

Sputum smear	Tubercular	Non-Tubercular	Total
Positive	30	0	30
Negative	54	16	70
Total	84	16	100

Table 2: Sensitivity and Specificity of Sputum smear for Pleural effusion

Sensitivity = 35.71%, Specificity = 100%, Positive predictive value = 100%,
Negative predictive value = 22.86%

ADA level	Tubercular	Non-Tubercular	Total
ADA >50 IU/L	82	2	84
ADA <50 IU/L	2	14	16
Total	84	16	100

Table 3: Sensitivity and Specificity of ADA Pleural fluid >50 IU/L

Sensitivity =97.6%, Specificity =87.5%, Positive predictive value =97.6%,
Negative predictive value = 87.5%

L/N ratio	Tubercular	Non-Tubercular	Total
>0.75	81	2	83
<0.75	3	14	17
Total	84	16	100

Table 4: Sensitivity and Specificity of L/N ratio for Pleural Fluid >0.75

Sensitivity = 96.42%, Specificity = 87.70 %, Positive Predictive Value = 97.59 %,
Negative Predictive Value = 82.35%

ADA and L/N ratio	Tubercular	Non-Tubercular	Total
ADA >50 IU/L and L/N >0.75	75	1	76
ADA <50 IU/L and L/N <0.75	0	13	13
Total	75	14	89

Table 5: Sensitivity and Specificity of ADA activity as an etiological marker for Pleural effusion in conjugation with L/N ratio (Lymphocyte/Neutrophil)

Sensitivity = 100 %, Specificity = 92.85 %, Positive Predictive Value 98.68%
 Negative Predictive Value = 100%

	Mean \pm 2SD
L/N ratio	1.2900 \pm 2.73746
ADA level	53.6229 \pm 9.22786
P Value	0.000

Table 6: Comparison of Mean between L/N ratio and ADA activity in Tubercular Pleural effusion