www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i11.217

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Significance of Adenosine Deaminase Levels in Diagnosis of Pleural Effusion

Authors
Dr Arjun Ramaswamy, Dr Prem Maurya, Dr Udaya Suresh, Dr CD Kulkarni, Dr Arif Ali
MGMIHS

Abstract
The present study was carried out in 71 patients in a tertiary care center with an aim to study the correlation of results of ADA test and lymphocyte proportion with clinical / radiological findings and results of other relevant laboratory investigations. Diagnostic utility of pleural fluid ADA levels in the diagnosis of tubercular pleural effusion was also determined and values of ADA in patients with tubercular pleural effusion and non tubercular pleural effusion compared. Incidence of tubercular pleural effusion was more common in 21 to 40 years age group and among sex distribution males were commonly affected compared to females. Fever, cough and chest pain were common presentation among the cases in the study group. Elevated ADA levels were seen among the cases who had cough in the study group. Positive Mantoux test result had significantly elevated ADA levels among the cases in study group. Presence of chest pain among the cases in study group had raised ADA levels. Cardiomegaly and oedema among the cases in study group did not have significant association with levels of ADA in the study group. Presence of cavitatory lesions and infiltrations in the lung had significantly elevated ADA levels among the study group. LDH, Sugar, TLC and lymphocytic proportions were significantly correlated with ADA levels among the cases in study.

Introduction

Pleural effusion is the presence of excessive quantity of fluid in the pleural space. Though it produces minimal symptoms, it should be considered as a sign of serious disorder. So, no effort should be left in arriving at specific diagnosis to give the most rational treatment. Effusion may be transudative due to abnormalities of hydrostatic, or osmotic pressures and exudative from increased permeability or trauma. Etiological diagnosis is often difcult to establish.

Although tuberculosis is the most common cause of effusion in developing countries like India, other causes should be excluded before labeling it as tuberculosis.

Investigation of pleural effusion demands pleural aspiration and biopsy, but its invasive nature and diffcult technique limits its practice. The pleural fluid is sent for measurement of proteins and glucose content, cytological and microbiological examination. Cytology and microbiology benefit from testing as large quantity of fluid as possible. A “diagnostic tap” of 10-20 ml of pleural fluid without a pleural biopsy is inadequate. In as many

as 20% cases of pleural effusions basic testing does not establish the diagnosis and even thoracotomy or thoracoscopy may not reveal the cause of effusion [2]

The diagnosis of pleural effusion is important because tuberculosis is normally a treatable cause of exudative lymphocytic pleural effusion. Other differential diagnosis of exudative lymphocytic pleural effusions are malignancy, fungal infection, sarcoidosis and connective tissue diseases. The primary diffculty in getting a diagnostic confirmation of tubercular pleural effusion is the identification of mycobacterium in the pleural fluid.

Many biologic parameters have been introduced. One such marker is adenosine deaminase (ADA) which has been proposed as a useful diagnostic tool. [3]

This study is intended to evaluate the adenosine deaminase level in tubercular pleural effusion and its value in differentiating tubercular from other causes.

Aims and Objectives

1. To evaluate utility of ADA test in cases of pleural effusion as a parameter indicating tuberculous etiology.
2. To correlate the lymphocytic proportion of pleural fluids with results of ADA test.
3. to attempt correlation of results of ADA test and lymphocyte proportion with clinical/radiological findings and results of other relevant laboratory investigations.

Materials and Methods

The study comprising of 71 patients was conducted after obtaining Ethical Committee clearance from the Institutional Ethical Committee at Tertiary care Centre. The diagnosis was based on clinical, radiological & laboratory findings.

Sputum AFB was done in patients w ho had cough with expectoration.

Estimation was carried out within 24 hours of sample collection. A total of 71 such pleural effusions were studied and all the patients underwent the following investigations.

a) Detailed clinical examination
b) Routine laboratory investigations – Hemoglobin, Complete blood count, Chest X ray, Mantoux test, sputum for AFB.
c) Diagnostic pleurocentesis for: Routine microscopy (total and differential cell count), Protein, Sugar, LDH, ADA estimation, cytology and ZNCF stain for AFB.

Inclusion Criteria

- Patients in the age group of 12-70 years
- Patients with clinico-radiological features suggestive of pleural effusion.

Exclusion Criteria

The pediatric age grou patients. The patients in whom pleural tapping was attempted but was a dry tap were excluded.

Methods

Adenosine Deaminase (ADA)

Adenosine deaminase (ADA) is an endogenous tissue enzyme, which is released into the serum in patients with different types of malignancies and infections including viral hepatitis, infectious mononucleosis and tuberculosis. In pleural fluid elevated ADA levels are very commonly associated with TB

ADAZYME comprises of:

a) R1 -ADAZYME - Enzyme Reagent (Lyophilized).
b) R2 - ADAZYME - Starter Reagent, ready to use.
c) R3 - ADAZYME - Buffer Reagent, ready to use.
d) C -ADAZYME - Calibrator (Lyophilized).

Principle: ADA

The ADA assay is based on the enzymatic deamination of adenosine to inosine, which is

converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H O) by xanthine oxidase (XOD). HO is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3- methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate a Quinone dye which is monitored in a kinetic manner.

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37º C.

Limitations

Assay is specific for ADA and has no detectable reaction with other nucleosides. Re reagent solution should be clear, and if turbidity is seen then the reagent may have deteriorated. If the sample ADA activity is greater than 200U/L, the sample should be diluted with normal saline. The result should be multiplied by the dilution factor.

Lactate dehydrogenase (LDH) levels in both pleural fluid and patient's serum were analyzed by an automated machine with the help of AU480 Beckman coulter analyzer.

Proteins in pleural fluid were analyzed by biuret method with the help of biochemistry analyzer.

Glucose in pleural fluid was analyzed by glucose oxidase method with the help of biochemistry analyzer. Blood glucose levels were estimated simultaneously.

The Total leucocyte count was counted with the help of Neubeur's chamber.

Differential Leucocyte count was done by micrcoscopically examining the Leishman and Hematoxylin and Eosin stained centrifuged deposit of pleural fluid.

Both Sputum and pleural fluid were examined microscopically for Acid Fast Bacilli by examining the Zeihl Nelson stained centrifuged deposits Tests for anti-HIV was done by Tridot test kits.

The tuberculin test was performed using 0.1 ml 5 TU PPD.

Patients were diagnosed positive for tuberculosis on the basis of lymphocyte predominance, high pleural fluid LDH, positive Montoux test, clinico-radiological features and clinical features suggestive of tuberculosis.

Results

Table 1: Age and sex wise distribution of cases in study group

Age (Yrs)	Male	Female	Total
< 20	4 (5.6)	2 (2.8)	6 (8.5)
21 – 30	9 (12.7)	7 (9.9)	16 (22.5)
31 – 40	16 (22.5)	1 (1.4)	17 (23.9)
41 – 50	10 (14.1)	0	10 (14.1)
51 – 60	8 (11.3)	2 (2.8)	10 (14.1)
>60	10 (14.1)	2 (2.8)	12 (16.9)
Total	57 (80.3)	14 (19.7)	71 (100)

The above table shows age and sex wise distribution among 71 cases in study group. Among 6 cases, 4 were males and 2 were females in <20 yr age group. Majority of subjects i.e. 17 were in age group 31 to 40 yrs with 16 were males and one female. 10 subjects were male among age group 41 to 50 yrs. Among 12 cases in more than 60 yrs age group, 10 were males and 2 were females. Among 16 cases in 21 to 30 yrs age group, 89 were males and 7 were females.

Table 2: Clinical feature wise distribution of cases in study group

Clinical feature		No of cases	Percentage (n=71)	ADA		MW test Z Value	P Value
				Mean	SD		
Fever	present	37	52.1	56.24	62.62	1.52	>0.05
	absent	34	47.9	38.11	28.14		
Cough	present	45	63.4	54.86	29.31	4.40	<0.0001
	absent	26	36.6	34.92	71.77		
Chest Pain	present	44	62	60.30	56.69	3.76	<0.0001
	absent	27	38	26.79	24.93		
Breathlessness	present	12	16.9	22.47	25.45	2.74	<0.01
	absent	59	83.1	52.66	52.02		
Oedema	present	10	14.1	21.13	25.31	2.84	<0.005
	absent	61	85.9	51.89	51.52		
Other	present	14	19.7	19.97	18.29	3.51	<0.0001
	absent	57	80.3	54.33	52.70		

The above table shows clinical feature wise distribution among 71 cases in study group. Cough and chest pain were most common clinical feature among 45 and 44 cases in the study group respectively. Fever was seen in 37 cases. Breathlessness, oedema were other symptoms in the study group.

Table 3: Correlation between lymphocytic proportion and ADA in study group

Correlation between ADA and	r Value	P Value
Lymphocytic proportion	0.25	<0.05

Pairs of quantitative variables as shown in this table were analyzed for correlation using Pearson's correlation coefficient (r). ADA was correlated with Lymphocytic proportion. Lymphocytic proportion and ADA was significantly associated with each other.

Table 4: Comparison of ADA according to consolidation and inftltrations /Cavitory lesion in study group

CXR finding			ADA		MW test Z Value	P Value
		n	Mean	SD		
Consolidation	Present	20	35.77	23.57	1.01	>0.05
	Absent	51	52.18	56.34		
Infiltrations/ Cavitatory lesion	Present	7	71.86	10.79	2.90	<0.005
	Absent	64	44.90	51.62		

The above table shows comparison of ADA according to consolidation and infiltration/ cavitatory lesions in the study group. Mean ADA level among consolidation present cases was 35.77 (S.D. of 23.57) and among cases without consolidation Mean ADA was 52.18 (S.D 56.34). This Mean ADA was analyzed quantitatively within groups as shown in above table. The Z value was 1.01, which was statistically not significant (p>0.05).

Mean ADA level among infiltration/cavitatory lesions present cases was 71.86 (S. D. of 10.79) and among cases without infiltration/cavitatory lesions Mean ADA was 44.90 (S.D 51.62). This Mean ADA was analyzed quantitatively within groups as shown in above table. e Z value was 2.90, which was statistically significant (p<0.005).

Tuberculosis Non tuberculous diseases ADA >34 U/L318ADA <34 U/L428

The sensitivity of the test is 88.57%.

The specificity of the test is 77.77%.

Table 5 Co-relation of ADA with Tuberculosis

	Tuberculosis	Non tuberculous diseases
ADA >34 U/L	31	8
ADA <34 U/L	4	28

Table 6: Comparison of ADA according to Montoux test in study group

Parameter	Montoux test						F Value	P Value
	0 – 5 (n=20)		6 – 10 (n=1)		>10 (n=13)			
	Mean	SD	Mean	SD	Mean	SD		
ADA	39.48	24.26	49.36	22.31	74.92	34.04	7.16	<0.0001

The above table shows comparison of ADA according to mantoux test result in the study group. This Mean ADA was analyzed quantitatively within groups as shown in above table. ie F value was 7.16, which was statistically significant (p<0.0001).

Discussion

The present study was carried out to study correlation of results of ADA test and lymphocyte proportion with clinical / radiological findings and results of other relevant laboratory investigations. The diagnostic utility of pleural fluid ADA levels in the diagnosis of tubercular pleural effusion was also determined and values of ADA in patients with tubercular pleural effusion and non tubercular pleural effusion compared.

The study was conducted in 71 adult patients who presented with clinic-radiological features suggestive of pleural effusion.

Among the 71 cases in the study group majority of them were males. Majority of cases were in age group of 21 to 40 yrs. (Table no 1)

Fever, cough and chest pain were the common symptoms among the cases in study group. Breathlessness and oedema were the other symptoms associated with cases in the study group. (Table no 2)

Fever was not significantly associated with mean ADA levels in the study group.

Mean ADA levels were significantly high in patients with cough as compared to those without cough. Sonone Kanchan K et al (2014) determine the exact role of ADA in TB patients with and without pleural effusion and in non-tuberculosis pleural effusion and to provide a clear picture of

ADA for early diagnosis & management of tuberculosis.4 Mean ADA levels with cases diagnosed pulmonary tuberculosis were 55.09 ±11.02 and in nontubercular pleural effusion mean ADA levels were 21.92±5.33. This mean difference was highly significant. This finding was similar with that in our study

Mean ADA levels were significantly high with chest pain as compared to those without chest pain in the study group. Mean ADA levels were significantly low among cases with breathlessness as compared to withot breathlessness in the study group. Zaysoe Wnna Halla Shwe, Soe Moe (2010) reported 108 patients with tuberculous pleural effusion and discussed the clinical features, radiological finding, bio chemical, cytological, and microbiological analysis of pleural fluid, hematological and biochemical profiles of serum in these patients. [5]

Common presentations were breathlessness (82.4%), Cough (81.5%), fever (80.6%) and night sweats (78.7%), Loss of appetite (74.1%), significant weight loss (72.2%) and chest pain (67.6%). Mean ADA levels were 73.9074 +/- 33.95. This finding was similar to our study. Mean ADA level were significantly low among cases with odema and other symptoms as compared to without odema in the study group.

Consolidation was not significantly associated with mean ADA levels in the study group (Table 4).

Mean ADA levels were significantly increased among cases with infiltration and cavitory lesions as compared to those without infiltration and cavitory lesions (Table 4). Cardiomegaly was not significantly associated with levels of mean ADA among the cases in study group.

Tuberculosis on chest X ray finding was significantly associated with increased levels of mean ADA among the study group. Mean ADA levels were raised among the cases with tuberculosis as compared to those without tuberculosis. Basu A, Chakrabarti I, Ghosh N and Chakraborty S (2012) evaluated the time tested relation with pleural fluid adenosine deaminase (ADA) level and cytological findings in the clinically suspected cases of tuberculous pleural effusion. Among suspected 44 tuberculosis cases 97.3% of cases had elevated ADA levels of > 70 U/L.[6] Sonone Kanchan K et al (2014) determine the exact role of ADA in TB patients with and without pleural effusion and in non-tuberculosis pleural effusion and to provide a clear picture of ADA for early diagnosis & management of TB. Pleural fluid ADA levels were significantly higher (p <0.0001) in pulmonary tuberculosis with pleural effusion (82.61+12.03) than in non tuberculous pleural effusion (27.72 +7.80).[4]

ME cell present had significantly raised mean ADA levels as compared to absent ME cell among the cases in study group.

Among the correlation between ADA and TLC, Polymorph, Eosinophil, Protein, sugar, proteins, LDH and Lymphocytic proportion, significant correlation was found with LDH, sugar, TLC and lymphocytic proportions. Y. C. Gary Lee, Jeffrey T. Rogers, Michael Rodriguez, Kent D. Miller and Richard W. Light (2001) studied the ADA levels in a variety of nontuberculous lymphocytic effusions and analyzed the relationships between ADA and conventional hematologic and biochemical parameters. There was no significant correlation between pleural fluid ADA levels and the total leukocyte (r = 0.08), differential lymphocyte (r = 0.18) or monocyte (r = - 0.18) counts and LDH (r=0.32). This finding resembled our study finding except for total leukocyte count. De Oliveira HG, Rossatto ER, Prolla JC (1994) combined use of both parameters Adenosine deaminase (ADA) and lymphocyte proportion was prospectively studied in 276 patients with pleural effusion. Using a cut-off level of 40 U/l at 37 degrees C (method of Giusti19) for ADA activity and lymphocyte proportion of more than 50%, the correct diagnosis of tuberculosis (sensitivity) was made in 90.7% (CI 87.3-94.1%) of 54 patients. The combined use of ADA activity determination and lymphocyte proportion is a

highly effcient diagnostic strategy.[8] Tunn Ren Tay and Augustine Tee (2013) determined the factors affecting pleural fluid ADA levels and to establish the optimal ADA levels for diagnosis of Tuberculous Pleural Effusion for different age groups. There was significant correlation between pleural fluid ADA and age, pleural fluid protein, LDH, and fluid absolute lymphocyte count which was similar finding with our study.[9]

Positive results on mantoux test had significantly higher levels of man ADA among the cases in study group as compared to negative results on mantoux test.

Conclusion

Incidence of pulmonary tuberculosis was more among males and in age group 21 to 40 years. Fever, cough, chest pain were common symptoms of pulmonary tuberculosis and along with breathlessness and odema. Positive Mantoux test had significantly high levels of ADA in pleural fluid. Raised ADA levels in pleural fluid were seen with cough, chest pain infiltration and cavitatory lesions. Biochemical markers correlated with ADA levels were LDH, sugar, TLC and lymphocytic proportions.

Reference

1. Richard W Light.Disorders of the pleura, mediastinum, diaphragm and chest wall. In: Kasper, Braunwald, Fauci, Haucer, Lingo, Jameson, ed. Harrison's principles of Internl Medicine.16th Ed. Vol,2,New York: Mc Graw Hill: 2005; 1565-1568.
2. Editorials: Tuberculous pleurisy and adenosine deaminase. thorax, 1995;50: 593-94.
3. Reechaipichitkul W, Kawamatawong T, Teerajetgul, et al. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion.South east AAsian J Trop Med Public Health, 2001; 32:383-9.
4. Sonone K K et al. Study of Adenosine Deaminase Levels in Patients of Pulmonary Tuberculosis with and Without Pleural Effusion. IOSR Journal of Dental and Medical Sciences.2014;13(1):30-37.
5. Zay Soe, Wunna Hla Shwe, Soe Moe. A Study on Tuberculous Pleural Effusion. International Journal of Collaborative Research on Internal Medicine & Public Health.2010; 2(3):32-48.
6. Basu A, Chakrabarti I, Ghosh N, Chakraborty S. A clinicopathological study of tuberculous pleural effusion in a tertiary care hospital. Ann Trop Med Public Health.2012; 5:168-72.
7. Lee YC, Rogers JT, Rodriguez RM, Miller KD, Light RW. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. Chest.2001;120(2):356-61.
8. De Oliveira HG, Rossatto ER, Prolla JC. Pleural fluid adenosine deaminase and lymphocyte proportion: clinical usefulness in the diagnosis of tuberculosis. Cytopathology.1994;5(1):27-32.
9. Tunn Ren Tay and Augustine Tee. Factors affecting pleural fluid adenosine deaminase level and the implication on the diagnosis of tuberculous pleural effusion:a retrospective cohort study. BMC Infectious Diseases.2013,13:546.