

Original Research Article

Study of adenosine deaminase levels in the diagnosis of tuberculous pleural effusion

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	International Archives of Integrated Medicine, Vol. 3, Issue 7, July, 2016. Copy right © 2016, IAIM, All Rights Reserved. Available online at http://iaimjournal.com/
	ISSN: 2394-0026 (P) ISSN: 2394-0034 (O)
	Received on: 03-07-2016 Accepted on: 13-07-2016 Source of support: Nil Conflict of interest: None declared.
How to cite this article: G. Anitha, R. Salma Mahaboob, G. Obulesu. Study of adenosine deaminase levels in the diagnosis of tuberculous pleural effusion. IAIM, 2016; 3(7): 353-357.	

Abstract

Background: Tuberculous pleurisy is thought to be the result of a delayed hypersensitivity reaction in response to the presence of mycobacterial antigens in the pleural space. This Immunologic reaction causes the stimulation and differentiation of lymphocytes, which release lymphokines, which in turn activate macrophages for an enhanced bactericidal effect.

Aim: To study the adenosine deaminase levels in the diagnosis of tuberculous pleural effusion.

Materials and methods: Present study was done in the Department of Biochemistry, Fathima Institute of Medical Sciences, Kapada. Parameters studied were total cell count, differential cell count, glucose, total proteins, lactate dehydrogenase, and adenosine deaminase.

Results: In our study, all the parameters were elevated in disease condition that was tuberculous pleural effusion compared to non-tuberculous pleural effusion.

Conclusion: Study of these parameters is not enough to know about the tubercular pleural effusion. The adenosine deaminase levels were significantly raised in pleural fluid in tuberculous pleural effusion than non tuberculous pleural effusion.

Key words

Tuberculous pleural effusion, Adenosine deaminase, Lactate dehydrogenase.

Introduction

Several reports have suggested that an elevated pleural fluid Adenosine Deaminase level predicts tuberculous pleuritis with a sensitivity of 90 to

100% and a specificity of 89 to 100%. There are several isoforms of Adenosine Deaminase but the prominent ones are adenosine deaminase1 and adenosine deaminase2. Adenosine

deaminase2 is the predominant isoform in Tuberculous Pleural Effusion [1].

Nowadays Tuberculosis is a serious worldwide problem because of AIDS and predicted spread of specific communicable diseases to normal population. Several types of Mycobacteria are responsible for disease in humans. Mycobacterium Tuberculosis is the cause of most infections. Complications of Tuberculosis are pleurisy with or without plural effusion, pneumothorax, empyema, tuberculous laryngitis, tuberculous enteritis and several others. However present study deals with tuberculous pleural effusion.

Pathogenesis of tuberculous pleural effusion

When a tuberculous pleural effusion occurs in the absence of radiologically apparent Tuberculosis it may be the sequel to a primary infection 6 to 12 weeks previously or it may represent reactivation Tuberculosis.

Tuberculous pleural effusion is thought to result from rupture of a subpleural caseous focus in the lung in to the pleural space which allows tuberculous protein to enter the pleural space and to generate the hyper sensitivity reaction responsible for most of the clinical manifestations [2]. Tuberculous Pleural Effusions are enriched with many potentially immune reactive cells and substances that comprise the vigorous local cell mediated immune response. Compared with peripheral blood; pleural fluid is enriched with T lymphocytes. The CD₄ (Helper induced) to CD8 (Suppressor/ Cytotoxic) ratio is 3: 4 in pleural fluid compared with 1: 7 in blood.

Clinical features

Include non specific cough, chest pain usually pleuritic in nature and hemoptysis, chills, fever, night sweats, fatigue and loss of appetite and loss of weight.

Adenosine Deaminase (ADA)

Adenosine Amino Hydrolase E.C. 3.5.4.4 is an enzyme involved in the catabolism of purine

bases, capable of catalyzing the deamination of adenosine forming inosine and deoxy adenosine to deoxy inosine in the process. Its main physiologic activity is related to lymphocytic proliferation and differentiation. The enzyme is widely distributed in animal tissues [3].

It is a glycoprotein containing glucosamine and galactosamine residues. The enzyme has a molecular weight of 32,500 to 33,000 daltons. It has an optimum P^H of 6.3. The structural gene is located on chromosome 20. The complete amino acid sequence of the enzyme consists of 363 amino acids. Secondary structure predictions assign adenosine deaminase to the alpha/ beta class of proteins [4].

Adenosine Deaminase is an inherited immuno deficiency accounting for about 25% of all cases of severe combined immuno deficiency (SCID) disease and is due to a lack of the enzyme. Adenosine Deaminase coded for by a gene on chromosome 20 [5, 6]. In humans, ADA, was mainly purified contaminant with Adenosine Deaminase binding protein and dipeptidyl peptidase IV, was not absorbed in adenosine-Sepharose, but was absorbed in IgG anti-ADA1-sepharose column and properties are compared with chicken ADA, Congenital deficiency of adenosine deaminase produces severe combined immuno deficiency (SCID).It is the first disease to be treated by somatic cell gene therapy [7].

Lactate dehydrogenase

(EC 1.1.1.27; L – Lactate; NAD reductase)

Lactate dehydrogenase is a hydrogen transfer enzyme which catalyses the oxidation of L-Lactate to pyruvate in presence of NAD as hydrogen acceptor. The reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate to lactate (P→L) [8, 9].

LDH – 1 migrates more quickly towards anode. Followed in sequence by the other fractions with LDH – 5 being slowest migrating one. Lactate dehydrogenase – 4 was eluted from the column with 10 ml of 0.02 M Tris – HCL buffer PH 6.3

containing 0.06M NaCl. Lactate dehydrogenase 3, 2 and 1 were eluted with 20 ml of the same buffer containing 0.1 M, 0.145 M and 0.24 M NaCl respectively [10].

Materials and methods

Selection of cases

Fifty clinically symptomatic cases of pleural effusion attending chest diseases outpatient department of Fathima Institute of Medical Sciences, Kadapa from January 2016 to June 2016 were included in this study.

Site of thoracentesis

Thoracentesis was attempted at one inter space below the spot where tactile fremitus is lost and the percussion note becomes dull [11]. The exact location for the thoracentesis attempt was just superior to a rib [12, 13].

Pleural fluid collection

The procedure was carefully explained to the patient and a signed consent forms obtained.

1st Step: The skin was cleaned with antiseptic solution. The sterile drape with the central hole was then taped to the patient's back and another sterile drape was placed on the bed.

2nd Step: - Anaesthetization of the skin, the periosteum of the rib and the parietal pleura was done [14]. 20/50 ml syringe was used containing 1 ml heparin to prevent clotting of the pleural fluid. The fluid was placed in EDTA treated tubes, Pleural fluid is tested for: Total Cell count, Differential cell count, Glucose, Total proteins, Lactate dehydrogenase, and Adenosine deaminase. All samples were cross checked in auto analyzer HITACHI 902 with precinorm and precipath in duplicates.

Results

Non-tuberculosis pleural effusion with various parameters, mean, SD, SEM and P values was as per **Table - 1**. Various Parameters with mean, SD, SEM and P values of tuberculosis pleural effusion was as per **Table - 2**.

Table - 1: Study of non-tuberculosis pleural effusion group (N=30).

Variables	Mean	SD	SEM	P Value
ADA	18.93	± 9.99	±1.823	<0.001
LDH	74.40	±64.54	±11.966	<0.001
T. protien	4.88	± 0.694	± 0.098	<0.001
Glucose	89.03	±36.64	±7.054	Not Significant
Cell Count	4986.67	± 1615.74	---	Not Significant

Table - 2: Study of cases of tuberculous pleural effusion group (N=70).

Variables	Mean	SD	SEM	P Values
ADA	160.86	±40.86	±5.778	<0.001
LDH	180.82	±194.99	±26.161	<0.001
T. Protein	4.87	± 0.694	± 0.098	<0.001
Glucose	108.98	±25.42	±3.594	<0.001
Cell Count	4062	±1244.55	---	<0.001

Discussion

The present study included eighty cases of pleural effusion, out of which fifty patients were with tuberculous pleural effusion, thirty patients were with non tuberculous pleural effusion. Increased Adenosine deaminase levels in pleural

fluid of tuberculous pleural effusion as compared to that of non tuberculous pleural effusion.

Other Studies also showed statistically significant elevation of adenosine deaminase levels in tuberculous pleural effusion compared with non-tuberculous pleural effusion.

Other studies also showed statistically significant elevation of dehydrogenase levels in tuberculous pleural effusion compared with non-tuberculous pleural effusion [15-20]. As per the results obtained the study was discussed under the following categories. Increased adenosine deaminase (ADA) enzyme levels in tuberculous pleural effusion than non tuberculous pleural effusion. Increased lactate dehydrogenase enzyme levels in tuberculous pleural effusion than non tuberculous pleural effusion [21-23]. There is not much of a difference in total protein levels of pleural fluid in tuberculous pleural effusion and non tuberculous pleural effusion. There is not much of a difference in glucose levels of pleural fluid of tuberculous pleural effusion and non tuberculous pleural effusion. There is not much of a difference in total count of pleural fluid - but in tuberculous pleural effusion there is lymphocytosis. In non tuberculous pleural effusion there is varying differential count some have monocytosis and some have lymphocytosis [24-32].

Conclusion

The adenosine deaminase levels were significantly raised in pleural fluid in tuberculous pleural effusion than non tuberculous pleural effusion. The lactate dehydrogenase enzyme levels were significantly raised in pleural fluid in tuberculous pleural effusion patients than in non tuberculous pleural effusion. There is a raise in total protein levels in pleural fluid in pleural effusion compared to non tuberculous pleural effusion but it is not statistically significant. There is a raise in glucose levels in pleural fluid in tuberculous pleural effusion compared to non tuberculous pleural effusion but it is not statistically significant. There is no difference in total count in pleural fluid of both tuberculous and non tuberculous pleural effusions but in tuberculous pleural effusion. There is lymphocytic exudate compared with non tuberculous where there are both lymphocytes and monocytes present. For understanding tuberculous pleural effusion other parameter investigations are required.

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