Estimation of Adenosine deaminase and proteinase inhibitory activity and their correlation with tuberculous effusion and other pleural effusion cases

Beenakumar K.[1], M.R.Laveesh[2,*], NVU Warrier[3], Parvathi K.[4], Jose Raj M.G[5]

[1]Assistant Professor, Dept. of Biochemistry, [2]Associate Professor, Dept. of Pharmacology, MMCH, Kozhikode
[3]Former Professor and HOD, Dept. of Pulmonary Medicine, [5]Professor and HOD, Dept. of Biochemistry, GMC, Kozhikode
[4]Professor and HOD, Dept. of Biochemistry, MES Medical College, Perinathalmanna

Corresponding Author:
E-mail: drlaveesh@gmail.com

Abstract

Introduction: Diagnosis of tuberculous effusion is difficult with the conventional, bacteriological, cytological and histopathological methods in our country. Adenosine deaminase, serum proteins, phospholipids show marked variation in tuberculous exudates.

Material and Methods: Twenty cases of pulmonary tuberculosis, 10 cases of malignant effusions and 8 cases of other para-pneumonic effusions visiting a tertiary care hospital were included in this study. After collecting blood and pleural fluid samples, Adenosine deaminase, Alpha-1 antitrypsin l, proteinase inhibitor activity, total lipid levels, phospholipid and Lipase activity were estimated.

Observation and results: Serum ADA levels in tuberculous effusion was 21.8+ 9.8 U/L(10-45.08) P <0.05 and that in tuberculous effusions after treatment was 12.2+4.4 U/L(6.2-20)P<0.05. Alpha-1 antitrypsin(mg%) levels in tuberculous effusion was 649.7+ 228.4 (365-949)P <0.05 and that in tuberculous effusions after treatment was 427.5 + 106.6(292-584)P<0.05.

Conclusions: Adenosine deaminase activity in serum and pleural fluid was found to be significantly elevated in pulmonary tuberculosis compared to the normal healthy subjects. ADA activity of serum and pleural fluid of malignant effusion cases(Bronchogenic carcinoma) and other parapneumonic effusions was found elevated compared to normal whereas the elevation in pulmonary tuberculosis was very high. So estimation of ADA in serum and pleural effusions may be a useful parameter for differential diagnosis of pulmonary tuberculosis from that of bronchogenic carcinoma and other parapneumonic effusions. ADA activity was found to be decreased in patients undergoing antituberculous treatment for a period of 6 weeks. So it may also be useful in the prognosis of the disease during treatment.

Keywords: ADA: Adenosine deaminase, $\alpha_1$antitrypsin, Tuberculosis, Proteinase inhibitory activity, Lipase, Phospholipid

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Introduction

Tuberculosis has probably been responsible for the greatest morbidity and mortality among the communicable diseases. Tuberculosis continues to be the major public health problem in India. As per the WHO estimates, about 100 million persons suffer from radiologically active pulmonary tuberculosis of which about 2.5 million would be sputum positive cases. The number of death is estimated to be nearly 500,000 every year[1].

Even though many methods have been developed for the diagnosis of tuberculosis, the problem is not yet solved. Uncomplicated initial stage tuberculosis often produces no significant clinical illness. A small caseous pulmonary focus may actually erode visceral pleura and extrude a small amount of liquid caseum. The immune response to such pleural contamination is a vigorous inflammatory reaction leading to formation of considerable pleural fluid. The pleural fluid is an exudate. The tuberculous nature of such pleural exudate is determined by culture or cytological or histopathological methods. The diagnosis is most often clinical, because smears of pleural fluid rarely reveal bacilli and even culture is positive in only 20- 25% of cases. Despite greater efforts to develop methods, there is no specific serological test to distinguish clinical tuberculosis from dormant tuberculosis[2]. Unfortunately tuberculosis is still the most important cause of exudative effusion in our country. Its detection and differentiation from other causes of pleural effusion is an important problem. In view of the difficulty in diagnosing tuberculous effusion by conventional, bacteriological, cytological and histopathological methods and the high incidence of tuberculosis in our country, the study is undertaken to confirm the role of adenosine deaminase assay in the diagnosis of tuberculous effusion, and its role in the prognosis of the disease as well.

The various components of serum proteins show marked changes in tuberulosis[3]. Electrophoretic analysis of serum proteins normal indicate that the values come back to normal with the clinical improvement[4]. There are reports of noticeable increase

in total cholesterol and decrease in the total lipids and phospholipid in tuberculosis[5,6].

Aims and Objectives

1. To confirm the usefulness of Adenosine deaminase assay in the diagnosis of tuberculous effusion and also to find out its role in prognosis
2. To find out any correlation of Alpha-1 antitrypsin levels between tuberculosis and other effusion cases
3. Estimation of total proteins to study the general aspects of protein metabolism in tuberculosis and other closely related chest diseases
4. Estimation of total lipid, phospholipid and lipase activity to correlate the occurrence of fatty infiltration in tuberculous and other effusion cases

Methodology

The study was conducted by the Department of Biochemistry in collaboration with the Department of Pulmonary Medicine, Government Medical College, Calicut. Twenty cases of Pulmonary tuberculosis, 10 cases of malignant effusions and 8 cases of other para-pneumonic effusions were included in this study.

Fifteen age and sex matched normal subjects formed the control group. All the subjects were in the age group of 20 to 70. Pleural fluid and blood were collected from all the patients. Seven cases were followed up for a period of 6 weeks of antituberculous treatment and then blood and pleural fluid were collected. The samples (Serum and pleural fluid) were stored at zero degree in the refrigerator. The estimation were carried within 72 hours of sample collection.

The following estimations were carried out in the serum and pleural fluid samples.

1. Adenosine deaminase activity
2. Alpha-1 antitrypsin level
3. Proteinase inhibitor activity using high molecular weight substrate-Casein
4. Proteinase inhibitor activity using low molecular weight substrate- BAPNA
5. Total protein estimation
6. Total lipid estimation
7. Phospholipid estimation
8. Lipase activity

The determination of adenosine deaminase activity in serum and pleural fluid was carried out after the colorimetric method of Galanti and Giusti[7] which is based on the indirect measurement of the formation of NH_3 produced when adenosine deaminase acts in excess of adenosine. Alpha-1 antitrypsin activity (Alpha-1 proteinase inhibitor) was estimated by the procedure followed by Hofman et al[8] and Witt and Lill H[9] as in Bergmeyer methods of enzymatic analysis.

Serum total proteinase inhibitory activity using high molecular weight substrate-Casein was measured following the method of Sujatha and Pattabiraman[10]. Serum total proteinase inhibitory activity using low molecular weight substrate-BAPNA was measured following the method of C.S. Sundaresh, A.R. Aroor and T.N.Pattabiraman[11]. Total protein estimation was done using the Biuret method .The method employed was modified method of Reinhold[12].

Total lipid estimation was done using the method of Phosphoric acid vanillin reaction given in the text book of Fundamentals of clinical chemistry by Norbert W. Tietz[13]. The term total lipid refers to all the lipid materials that can be extracted readily from a specimen.

Phospholipid was estimated following the procedure of Connerty et al[14] which used the Gomori's method to determine inorganic phosphorous. Lipase (Triacyl glycerol acyl hydrolase) activity was measured using the method of Cherry and Crandall[15]described in the Micro techniques of Clinical Chemistry by Samuel Natelson.

Results

Table 1: Serum values of ADA in the various study populations

	Normal	Pulmonary Tuberculosis	Malignant effusion	Other Para-pneumonic effusion	Tuberculous effusion after treatment
Range (U/L)	`2.3-7.0	10-45.08	7.1-2.8	5.2-9.1	6.2-20
Mean (U/L)	4.6	21.8	10.3	6.9	12.2
S.D. (U/L)	1.4	9.8	4.4	1.2	4.4

Table 2: ADA values in pleural fluid in the various study populations

	Normal	Pulmonary Tuberculosis	Malignant effusion	Other Para-pneumonic effusion	Tuberculous effusion after treatment
Range(U/L)	`21.	10-45.08	7.1-2.8	5.2-9.1	6.2-20
Mean(U/L)	4.6	21.8	10.3	6.9	12.2
S.D.(U/L)	1.4	9.8	4.4	1.2	4.4

Table 3: Values of ADA, Alpha-1 antitrypsin, Proteinase inhibitors and total protein in control and 4 classes of patients' serum expressed as Mean+SD

Classes	No. of cases	ADA(U/L)	Alpha-1 antitrypsin (mg %)	Proteinase inhibitor using Casein	Proteinase inhibitor using BAPNA	Total protein (gm %)
Normal	15	4.6+ 1.4 (2.3-7.0)	231.6+ 26.0 (196-292)	23.6+ 1.8 (17.5-32.2)	31.6+ 8.3 (17.5-40.2)	7.0+ 0.9 (5.6-7.8)
Tuberculous pleural effusion	20	21.8+ 9.8 (10-45.08) P <0.05	649.7+ 228.4 (365-949) P <0.05	41.7+ 11.6 (21.5-87.13) P <0.05	35.3+ 11.9 (11.1-50.0)	7.4+ 0.9 (4.6-8.6)
Malignant pleural effusion	10	10.3+ 4.4 (7.1-20.8) P <0.05	525.6+ 252.9 (292-876) P <0.05	34.3+ 15.4 (21.5-71.13)	33.3+ 8.9 (12.12-46.6)	6.8+ 1.4 (4-8.7)
Other parapneumonic pleural effusion	8	6.9+ 1.2 (5.2-9.1) P<0.05	261.8+ 39.6 (196-292)	25.1+ 3.4 (19.6-30.5)	37.3+ 5.7 (28.5-45.2)	6.4+ 0.5 (5.8-7.1)
Tuberculous effusions after treatment	7	12.2+ 4.4 (6.2-20) P<0.05	427.5+106.6 (292-584) P<0.05	35.3+ 14.2 (22.5-67.5)	29.5+ 4.2 (24.5-35.2)	6.5+ 0.9 (4.5-7.4) P<0.05

Table 4: Levels of total lipid, phospholipid and lipases in control serum and 4 classes of patients' serum expressed as mean +SD

Classes	No. of cases	Total lipid mg%	Phospholipid (mg%)	Lipase Comfort Unit
Normal	15	443.5+ 101.2 (235.8-601.1)	233.1+31.7 (183.5-301.1)	0.4+ 0.1 (0.25-0.7)
Tuberculous pleural effusion	20	427+ 147.7 (180-741.1)	191.1+ 26.3 (100.6-262.1) P <0.05	0.4+ 0.1 (0.25-0.7)
Malignant pleural effusion	10	432.8+204.1 (150-950)	268.8+ 138.2 (105.5-510)	0.4+ 0.3 (0.25-0.7)
Other parapneumonic pleural effusion	8	425.1+ 64.4 (321.9-521.6)	180.3+ 28.3 (116.5-220.6)	0.2+ 0.1 (0.25-0.45)
Tuberculous effusions after treatment	7	417+ 142.6 (252.5-694.6)	209 + 71.4 (125.5-346.5)	0.3+ 0.09 (0.3-0.5)

Table 5: Levels of ADA, Alpha-1 antitrypsin, Proteinase inhibitors and total protein in pleural fluid of 4 classes of patients' expressed as Mean+SD

Classes	No. of cases	ADA(U/L)	Alpha-1 antitrypsin (mg%)	Proteinase inhibitor using Casein	Proteinase inhibitor using BAPNA	Total protein (gm%)
Tuberculous pleural effusion	20	37.3+15.1 (21.5-83.1) P <0.05	377.4+181 (196-365) P <0.05	43.7+ 16.1 (21.1-84.9)	40.4+ 14.3 (18.1-67.2)	6.6+ 1.4 (4.5-8.7) P <0.05
Malignant pleural effusion	10	13.6+ 6.5 (10.8-25.8) P <0.05	255.5+ 99.3 (73-365)	26.2 + 10.1 (11.8-44.5)	41.3+13.9 (29.6-64.1)	4.7+ 1.5 (2.5-7.1) P <0.05
Other parapneumonic pleural effusion	8	12.9+ 2 (9.8-16.5) P<0.05	207.5+11.5 (196-219)	21.6+ 2.7 (18.6-26.4)	34+ 5.7 (26.5-41.2)	4.3+ 0.7 (3.2-5.2)
Tuberculous effusions after treatment	7	24.1+10.9 (16.6-50.1) P<0.05	306 +74 (196-365)	27.1+ 15.8 (22.5-50.9)	34.1+ 12.9 (18.1-53.7)	5.3+ 1.4 (3.2-7.6)

Table 6: Levels of total lipid, phospholipid and lipases in pleural fluid in 4 classes of patients' serum expressed as mean +SD

Classes	No. of cases	Total lipid mg%	Phospholipid (mg %)	Lipase Comfort Unit
Tuberculous pleural effusion	20	301.9+ 103.9 (180.1-441.4) P<0.05	176.1+ 41.3 (102.5-250.6) P<0.05	0.4+ 0.1 (0.1-0.7)
Malignant pleural effusion	10	258.8+ 120.5 (180-402.5) P<0.05	243.7+ 57.5 (102.2-340.5)	0.4+ 0.2 (0.2-0.7)
Other para pneumonic pleural effusion	8	403.2+ 46.7 (340.5-450.08)	153.6+26.4 (106.7-181.1)	0.3+ 0.1 (0.2-0.4)
Tuberculous effusions after treatment	7	320.1+184.5 (205.6-421.4)	183 +27.5 (139.4-220.5)	0.3+ 0.1 (0.25-0.45)

Discussion

In the present study the ADA level in serum and pleural fluid were higher in patients with tuberculous effusions than in malignant pleural effusions and in other parapneumonic effusions. The study conducted by Baldev Raj et al concluded that the ADA levels in the serum and pleural fluid of patients with tuberculous effusion was significantly elevated than the control group and other class of patients. This is in conformity with our results. However in the studies done by Straub et al and Letnansky and Seelich et al showed that ADA level was increased in patients with malignant tumours.

The studies conducted by Sulochana et al , Piras et al and Hitoshi Sanada et al concluded that the ADA activity were significantly elevated in tuberculous effusions when compared to malignant effusions and other para-pneumonic effusions. The results of our study closely agree with the above studies.

ADA is an enzyme involved in the purine catabolism and seems to be produced to a greater extent by more differential or activated T lymphocytes. Its serum activity is higher in disease where cellular immunity is stimulated. The increase in its activity in tuberculous pleurisy and empyema were due to T cell mediated immunity.

In the treatment group (Patients undergoing treatment with Rifampicin, INH and Ethambutol for a period of 6 weeks) a significant decrease in ADA activity was noted (P value <0.05).So it can be concluded that increase in ADA in pleural fluid and serum is a protective mechanism against the infections.

Human serum contains a variety of proteins which are capable of inhibiting trypsin, chymotrypsin elastase and other proteolytic enzymes. Of the known proteinase inhibitors, α_1 antitrypsin appears to be the important one. The proteinase inhibitors are considered to play a protective role against proteolytic action of pancreatic and intracellular proteinases. α_1 antitrypsin qualitatively important in lung tissue compared to other tissues. Concentration of α_1 antitrypsin in serum seems to be elevated in patients with inflammatory and neoplastic diseases.

In the present study we found an increase in α_1 antitrypsin activity in serum samples of tuberculous effusion cases compared with the normal control (P <0.05).An increased level was noticed in malignant effusion cases and in other parapneumonic effusions also. The values of α_1 antitrypsin was found decreased in cases followed up after 6 weeks of treatment.

In pleural fluid α_1 antitrypsin level was found to be increased in tuberculous effusions which was less than the corresponding values in serum. This is in concordance with the study conducted by Sulochana et al. The decreased activity after treatment was not so significant, probably due to protein released after infections, continues to remain in the circulation for some time.

Highly raised activity of α_1 antitrypsin in serum in tuberculous patients compared to malignancy and other type clearly demonstrate the usefulness of estimating α_1 antitrypsin in the diagnosis of tuberculous infection. This elevation of α_1 antitrypsin may be beneficial in tuberculosis by limiting proteolysis. According to Dixit et al and Yemul et al the α_1 antitrypsin values were significantly higher than in controls(P<0.05) which is in conformity with our study. None of the patients of tuberculous effusion in the present study suffered from a deficiency of α_1 antitrypsin. The raised level of this protease represent an acute phase reaction to the infection.

Proteinases have basic functions in digestion and in regulating proteolytic mechanisms such as peptide hormone release, coagulation and complement activation. They also form pathogenic factors in many diseases including tumours, inflammatory diseases, pulmonary emphysema, glomerulonephritis, acute pancreatitis and muscular dystrophy. Proteinase inhibitory activity in pulmonary tuberculosis was found to be increased in serum as well as in pleural fluid. The percentage inhibition calculated in method using "Casein" was found significantly increased in tuberculous patients serum when compared with normal controls(P<0.05). But there is slight increase in proteinase inhibitory activity using "BAPNA" in

pleural fluid of malignant effusion cases. The increase in values of proteinase inhibitors in serum and pleural fluid of malignancy are not very significant compared to the values of α_1 antitrypsin.

Previous studies indicate that various components of serum proteins undergo very marked changes in tuberculosis. In the present study total serum proteins was found to be slightly increased. In malignant effusions, the total proteins in serum was found to be lowered when compared to the normal controls and tuberculous patients. This may be due to immunodeficiency seen in malignant conditions. Total protein in other parapneumonic effusions and tuberculosis after treatment was also found to be lowered. A significant fall of total protein in serum was observed after 6 weeks treatment which is in conformity with the study done by Indira and Sirsi et al. In pleural fluid, the estimation of total protein was conducted to find the nature of the fluid-exudate or transudate. The values above 3gm/dl was considered to be exudative in nature. In tuberculous infection, the fluid is mainly exudative in nature.

In the present study, the protein content of the pleural fluid was found to be significantly decreased on comparison with normal serum level which is in concordance with Sulochan a et al. The results indicate that tuberculous effusion fluids are highly exudative in nature when compared to the malignant effusion fluid and effusions due to other parapneumonic infections. After treatment, the total proteins in the pleural fluid was found to be decreased.

Multiple disturbances in the metabolism of lipids in liver in experimental animals have been reported. In our study, the total lipid in tuberculous patients was found to be lower than the control serum lipid. This finding was similar to the report of Serizawa et al and Seth et al. A decrease in total lipid level was also observed in serum of patients with malignant effusions. The total lipid in pleural fluid was found decreased significantly in tuberculous effusions and malignant effusions ($P<0.05$). This fall may be due to increased demand of lipids for the growth of mycobacteria.

Mycobacterial cell wall contains phospholipids in large quantities. Hence it is pertinent to investigate this fraction. In the present study, the serum phospholipid content in tuberculous effusion cases was found to be significantly lower than the controls which is in conformity with the study of Seth et al. In malignant effusions, the serum phospholipid was little elevated than the control. Lowered level of phospholipid in tuberculosis may be due to consumption of phospholipid by the mycobacteria for its growth, but this is not the case in malignancy. The decrease in phospholipid in serum may also due to inhibition of transacylase reaction.

In pleural fluid the phospholipid content was found to be lower than the serum values found in the cases of tuberculous effusion and malignant effusion. The phospholipid content was found to be elevated after treatment both in pleural fluid and in serum which was not statistically significant.

Lipases are enzymes that hydrolyse the long chain fatty acid triglycerides. In the present study the lipase activity recorded in comfort units in serum and pleural fluid in various classes of patients shows no significant change.

Conclusion

Adenosine deaminase activity in serum and pleural fluid was found to be significantly elevated in pulmonary tuberculosis compared to the normal healthy subjects. ADA activity of serum and pleural fluid of malignant effusion cases(Bronchogenic carcinoma) and other parapneumonic effusions was found elevated compared to normals whereas the elevation in pulmonary tuberculosis was very high. So estimation of ADA in serum and pleural effusions may be a useful parameter for differential diagnosis of pulmonary tuberculosis from that of bronchogenic carcinoma and other parapneumonic effusions. ADA activity was found to be decreased in patients undergoing antituberculous treatment for a period of 6 weeks. So it may also be useful in the prognosis of the disease during treatment.

α_1 antitrypsin values in serum and pleural effusion fluids was found to be significantly elevated pulmonary tuberculosis. In malignant effusions and other parapneumonic effusions, α_1 antitrypsin values were not very much elevated when compared to pulmonary tuberculosis. Its value was found to be decreased along with treatment in pulmonary tuberculosis. So the estimation of α_1 antitrypsin may be useful along with ADA estimation in the diagnosis and prognosis of pulmonary tuberculosis.

The proteinase inhibitory activity as judged by high molecular weight substrate or by low molecular weight substrate are not very useful in the diagnosis and prognosis of pulmonary tuberculosis, malignant effusions or other parapneumonic effusions.

Serum total protein was found to be slightly elevated in pulmonary tuberculosis, whereas in malignant effusions, the total protein was found to be slightly decreased compared to the controls. In other parapneumonic effusions, total protein in serum was not altered compared to normal.

Serum Phospholipid was significantly decreased in pulmonary tuberculous cases, compared to the malignant effusion cases and other parapneumonic effusion cases and healthy controls.

Phospholipid content of the pleural effusion fluid were found to be significantly decreased in comparison with normal serum or in comparison with effusions of bronchogenic carcinoma and other parapneumonic effusions.

Total lipid in serum was found to be lowered when compared to normal healthy subjects in pulmonary

tuberculosis. In bronchogenic carcinoma and in other parapneumonic effusions. Total lipid in tuberculous effusion was found to be significantly decreased than compared with the normal serum values. In malignant effusion fluid also there is significant decrease in the total lipid level when compared with the normal control serum.

Serum and pleural fluid lipase activity was not altered in pulmonary tuberculosis, malignant effusions and other parapneumonic effusions.

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