STUDY OF ADENOSINE DEAMINASE ACTIVITY AS A BIOCHEMICAL MARKER OF CELL MEDIATED IMMUNITY IN TUBERCULOUS PLEURAL EFFUSION

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ABSTRACT

The present study conducted on 100 patients with the aim that ADA estimation is a simple cheaper and quicker biochemical test and could provide additional supportive evidence for the diagnosis of tuberculous pleural effusion in clinically suspected cases and will therefore help in early institution of therapy to these patients. The data was collected and interpreted at department of Physiology and Department of Biochemistry, Jhalawar Medical College, Jhalawar, Rajasthan.

Keyword: *ADA, Biochemical Test, Tuberculous Pleural Effusion.*

INTRODUCTION

Pleural effusion is a common chest problem, yet it is difficult to establish the a etiological diagnosis in as many as 20% cases in spite of good history, thorough clinical, radiological, full examination of aspirated fluid and pleural biopsy (Kataria and Imtiaz; 2001). It affects millions of people not only in India but also all over the world. It may affect the lungs and all other systems. This infection is caused by Mycobacterium Tuberculosis. It is second largest infectious disease all over the world after the HIV. So there is a need of simple, rapid and reliable diagnostic test to establish the aetiology of pleural effusion. Considering this a prospective hospital based study was designed to compare pleural fluid adenosine deaminase level is establishing the diagnosis of tubercular pleural effusion (Verma et al; 2008). Adenosine deaminase (Spencer et al; 1968) is an enzyme widely distributed in human tissues, particularly T-lymphocytes. It catalyzes the irreversible hydrolytic deamination of adenosine to produce Inosine and Ammonia (Martin et al; 1976.). Its plasma activity is high in diseases where cellular immunity is impaired. Thus, increased level of ADA is found in various form of tuberculosis, making it as a marker particularly for pulmonary tuberculosis. (Giblett et al; 1972)

Therefore, increased level of ADA is found in various forms of tuberculosis making it a marker for the same. Hence in the present study, ADA activity was studied for easy diagnosis of tuberculous pleural effusion. The present work was done in the department of Physiology and Department of Biochemistry, Jhalawar Medical College, Jhalawar.

MATERIALS AND METHODS

The study was conducted on 100 cases of tuberculous pleural effusion who volunteered for the study. The study population consisted of both 50 male and 50 female with an average range of 30 to 70 years. This study was conducted in department of Physiology, Biochemistry and T.B. and Chest Department, Jhalawar Medical College and SRG Hospital, Jhalawar (Rajasthan). The study subjects were selected from patients coming to OPD and admitted in SRG Hospital and Jhalawar Medical College, Jhalawar. Permission for present study has been taken from ethical committee of Jhalawar Medical College and SRG Hospital, Jhalawar.

Diagnostic criteria for Pleural Effusion Tuberculosis:

Physical examination

Cloudy, Clear, bloody, serous, yellow

Biochemical Examination of Pleural Fluid

I. Raised protein > 50% serum proteins.
II. Glucose < 60 mg /dL.
III. Cell count > 500 cumm predominant lymphocytes cells.
IV. ADA > 40 U / L. (cut off value)

Hematological Examination

Estimation of Leucocyte count, hemoglobin and ESR was done by employing standard methods.

Assessment of nutritional status

Anthropometric measurements were done and Body mass index was calculated as per the standard procedure (Vasudevan et al, 2013).

Statistical Analysis

This study is prospective case control study. Standard statistical analysis was performed for evaluation of data such as student "t" test and ANOVA test with the help of statistical package social science (SPSS).

RESULTS AND OBSERVATIONS

The study comprised of 60 normal healthy controls aged 18- 48 years and newly diagnosed 40 cases of tuberculous pleural effusion who volunteered for the study. The study population consisted of 50 men and 50 women with an average age range of 30-70 years. The adenosine deaminase was measured and correlated with various parameters. Table 1 depicted, the low mean hemoglobin levels in both groups reflect the poor nutritional status of the patients under study. On the other hand the mean ESR values recorded were on higher side supporting the tuberculous pathology. Total leucocyte count was in the normal range in both groups.

Data in Table 2 depicted that statistical analysis revealed a significant comparison of biochemical and leukocyte count based on ADA levels in tuberculous pleural effusion patients and control group with Determination (mean + S.D.) of ADA (U/l), Total Protein concentration (gm/dl) and Glucose (mg/dl) were analyzed in pleural fluid patients (i), control group (ii). comparison of both groups case and control ADA (U/l), total Protein concentration (gm/dl) and glucose (mg/dl) were performed for calculation of t value and p value (I vs II) and it were found significant.

Determination (Mean $\pm$ S.D.) of Pleural fluid ADA (U/l) and total protein concentration (gm/dl) were analyzed in two groups (I. n = 60, II. n = 40) the number of total cases were 50 (Table 3). To differentiated transudate from exudates the ratio of pleural fluid and serum protein was calculated. Determination (Mean $\pm$ S.D.) of Pleural fluid ADA (U/l) and Glucose concentration (mg/dl) were analyzed in total 60 cases (Table 4).

In Table 5, the Mean $\pm$ S.D. of ADA (U/l) and Total Leukocyte (Count / cumm) in Tuberculous pleural fluid of patients (group = I n =60, group = II n =40, entire series n = 50) were categorised. In group I Total Leukocyte Count / cumm < 500 AND in group II it was >500.

Table No.1: Hematological Profile of the Study Population

S.No.	Parameters	Control Group (n= 60)	Tuberculous Pleural Effusion (n=40)
1.	Hb (gm/dL) Mean + S.D. Range	 14.82$\pm$1.34 12.9 -16.0	 14.7$\pm$1.75 10 – 16.2
2.	ESR (at the end of 1st hour in mm) Mean + S.D.	 5.88$\pm$1.27	 20.94$\pm$6.49

	Range	4 - 8	9 – 40
3.	TLC / cumm Mean + S.D. Range	 7639±1472.5 5100 - 10800	 7772±1431.1 5100 - 10800
4.	Weight (kg) Mean + S.D. Range	 56.66 ±7.18 43-76	 50.02±8.2 38 – 69
5.	ADA Activity Mean + S.D. Range	 16.5±3.53 9 – 13	 78.92±12.50 70 – 110

Hb - Hemoglobin
ESR - Erythrocyte sedimentation rate
TLC - Total leucocyte count
ADA- Adenosine Deaminase Activity

Table No. 2: Comparison of biochemical and leukocyte count based on ADA levels in tuberculous pleural effusion patients and control group

	Mean ± SD		
	ADA (U/l)	Protein (g/dl)	Glucose (mg/dl)
Pleural fluid Patients (I)	89.92±12.50	6.25±0.80	37.39±4.80
Control Group (II)	14.5±3.52	5.77±0.67	73.98±8.22
't' value I Vs II	34.04	-10.25	-33.83
'p' value I Vs II	0.00	0.00	0.00
Significance	Significant	Significant	Significant

Table No. 3: Pleural fluid ADA level in relation to pleural fluid proteins in tuberculous pleural effusion patients (n = 50)

Group	**Pleural Fluid Protein g/dl**		**ADA U/L**
	Category	**Mean ± S.D.**	**Mean ± S.D.**
Entire series (n = 50)	-	5.25±0.80	89.92± 12.50

Table No. 4: Pleural fluid ADA level in relation to pleural fluid glucose in tuberculous pleural effusion patients

Group	**Pleural Fluid Glucose mg/dL**		**ADA U/L**
		Mean ± S.D.	**Mean ± S.D.**
Entire series (n = 50)		38.39 ± 4.80	89.92± 12.50

Table No. 5: Tuberculous pleural fluid ADA levels in relation to leukocyte count

Group	**Total Leukocyte Count / cumm**		**ADA U/L**
	Category	**Mean ± S.D.**	**Mean ± S.D.**
I (n =10)	< 500	401.0 ±74.30	81.1 ±15.21
II (n =40)	> 500	777.5 ±161.68	79.62 ±11.94
Entire series (n = 50)	–	702.2±212.04	75.32 ± 40.12

DISCUSSION

Tuberculosis occurs worldwide and is rampant in many countries. Through curable, its infection is on the rise. The most specific test is the positive bacterial culture of a patient's sputum sample. This is cumbersome and time consumption. X-rays, smears for AFB and tuberculin tests though comparatively rapid are not conclusive. The sign and symptom of tuberculous pleural effusion were fever, cough, weight loss, chest pain, breathlessness and loss of appetite. Generally physical signs and clinical symptoms do not positively help for accurate diagnosis of tuberculous pleural effusion. Adenosine Deaminase (ADA) is an enzyme widely distributed in mammalians tissues, particularly in T Lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. Though ADA is also increased in various infectious diseases like

infectious Mononucleosis, Typhoid, Viral Hepatitis, initial stages of HIV, and in cases of malignant tumors, the same can be ruled out clinically. We have studied 100 cases of male and female between age 30 – 70 years in patients coming to SRG Hospital and Jhalawar Medical College, Jhalawar.

Table 1 showing in present study hematological data of healthy controls group and patients of tuberculous pleural effusion. However, in patients group it was found (19.59±2.10) and showed chronic energy deficiency or underweight. In Tuberculous pleural effusion patients were more nutritionally compromised as compared to healthy control group Zay soe et al 2010.

Anemia is a condition in which there is reduction in the hemoglobin content of blood or in the number of red blood cells or both or defective maturation of the red blood cells (Dacie and Lewis 2001). Hemoglobin level (gm %) in blood sample of Tuberculous pleural effusion subjects and in blood of healthy control groups were estimated. Both the parameters revealed nutritional status. In healthy subjects it was found within normal limits with mean ± SD was 13.82±1.34 and range was found 11.9-16.0 (Table 2). In present study lower range of hemoglobin demonstrated poor nutritional status and reduced cell mediated immunity. Similar study reported in literature (Zay Soe et al 2010 and Agarwal 2012).

The mean value of Erythrocyte sedimentation rate (ESR) at the end of 1st hour in healthy control group and Pleural effusion of Tuberculosis patients were performed and found to be 4.88±1.27 with range 3-7 and 21.94±6.49 and range 10-40 respectively. The increased level of ESR in study group of pleural effusion was due to mycobacterium tuberculin infection. We have compared our results with other workers which showed similar findings (Motoki Sakuraba et al 2009 and Zay Soe et al 2010) (Table 3).

The white blood cell counts (WBC) determines the number of Leukocytes per cm of whole blood the range and mean value of total leukocyte counts in both the groups (control and case) were within normal limits in blood (5100-10800 / cumm) (Table 5). Alteration in total whites cell count indicates the degree of response to a pathological process but it is not specifically diagnostic for any one disorder (Selkurt 1982).

Adenosine Deaminase (ADA) is an enzyme in the purine salvage pathway required for converting adenosine to inosine. ADA levels are ten times higher in lymphocytes than in erythrocytes, particularly T lymphocytes (Ramgopal S and Reem GH, 1982). However in control group the ADA cut off value was less than 30 U/l with range 12-19 U/l. (Table 4). We have been compared ADA level in both case and control groups ($t = 34.04$, $p = 0.00$) which revealed significant ADA level in present study (Table 4). Increased values of ADA in tuberculous pleural effusion have been studied by several research workers. These results were further confirmed in our study. Our results showed that ADA is significant marker in diagnosis of tuberculous pleural effusion. Our findings were similar with reports studied by other researchers (Agarwal 2012). Tuberculous pleural effusion is the result of a cell mediated immune response to the presence of Mycobacterium tuberculosis and is characterized by the accumulation of activated T lymphocytes and macrophages in the pleural space. ADA is reported to be associated with severe form of combined immune deficiency and it's responsible for an increase in toxic nucleotides that prevent the differentiation or proliferation or both of T lymphocytes and thus a normal immune function mediated by cells. The raising of the levels of ADA activity under antigenic stimulation shows the importance of this enzyme in the rapid proliferation of cells in order to prevent the accumulation of toxic metabolites. Therefore, an increased ADA activity is present in several circumstances in pleural effusion of a tuberculous nature (Agarwal 2012).

CONCLUSION

Determination of Adenosine Deaminase (ADA), Glucose and Protein is important in diagnosis and medical management of tuberculosis disease in pleural fluid. In present study we have

estimated ADA level, glucose, protein and hematological parameters in pleural fluid of tuberculosis and serum of healthy control group in Jhalawar area of Hadoti region of Rajasthan. In our study, we had correlated ADA, glucose and protein, Hematological parameters and cytological parameters in diagnosis of tuberculous pleural effusion. Suitable statistical tool was applied to find out the significance of this study in relation with diagnosis, prognosis and severity of tuberculous disease in pleural effusion.

100 cases and control of different case groups male and female were studied in this study. Lower level of hemoglobin in our study in Tuberculous Pleural effusion cases demonstrated Anemia due to malnutrition. Increased level of ESR showed Mycobacterium Tuberculosis infection in patients of tuberculous effusion. Total Leukocyte count was within normal limits in both case and control in study population. However Differential leukocyte counts particularly lymphocyte count in pleural fluid of tuberculous effusion was significantly increased.

In present study ADA estimation was planned as screening test with cut off value >40 U/l for the diagnosis of Tuberculous Pleural effusion. In our study we concluded that the best cut off level of ADA activity was tested mean $\pm$ SD 79.92$\pm$12.50 and suggested that ADA estimation was a test for the diagnosis of tuberculous pleural effusion. From this study we concluded that using >40U/l as the cut off value of ADA estimation in Tuberculous Pleural effusion confirms the high sensitivity (88%) and specificity (90%) of ADA test with glucose and protein level in tuberculous pleural effusion not only simple, rapid and cheaper procedure in India where there is a high incidence of Tuberculosis but also significant in diagnosis, prognosis and medical management of Tuberculous Pleural Effusion.

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