ADENOSINE DEAMINASE ANALYSIS IN TUBERCULOUS PLEURAL EFFUSION

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Abstract:
Tuberculosis is a major cause of pleural effusion, in which pleural fluid usually has lymphocytic and exudative characteristic. Accurate and early diagnosis of tuberculous pleurisy is essential for its correct treatment and management in clinical practice. Adenosine Deaminase is an enzyme that increases in Tuberculosis (TB) because of the stimulation of T cell lymphocytes by mycobacterial antigens. The current methods for the diagnosis of Tuberculosis do not provide enough sensitivity and specificity. Aims and objectives-Aim of the study is to evaluate the ADA activity in patients with tuberculous pleural effusion and transudative pleural effusion and to find out the diagnostic role in tuberculous pleurisy. Materials and Methods-One hundred cases admitted in the medical ward on account of pleural effusion were selected. As per Lights criteria cases are classified in to exudate and transudate. The final diagnosis was based on standard diagnostic criteria. For TB pleural effusion, the following criteria was adapted ctice.

1. Bacteriological confirmation of presence of Mycobacterium tuberculosis. (direct smear or culture).
2. Radiological finding consistent with tuberculosis exclusion of other clinical consideration.
3. Clinical presentation consistent with tuberculosis (with exclusion).

Group I includes 50 cases of Tuberculous pleural effusion and Group II includes 50 cases of transudative pleural effusion. ADA was estimated in pleural fluid in both the groups. ADA activity was measured in pleural fluid by the Guisti and Glantis method using semi autoanalyser.

Results- The mean and standard deviation of ADA level in pleural fluid was significantly high in group I (102.86 plus or minus 22.61) when compared to group II (16.48 plus or minus 3.02) and the p value was statistically significant.

Conclusion- Our study supports the view that pleural fluid ADA estimation is a very useful biomarker in establishing an accurate diagnosis in tuberculous pleural effusion. In addition it is a simple, rapid and an inexpensive investigation that could be a complimentary
INTRODUCTION:
The pleural space lies between the lung and the chest wall. It normally contains a very thin layer of fluid 8.4± 4.3 ml\(^1\). Pleural effusion results when there is excess pleural fluid formation or decreased fluid removal by the lymphatics. It is one of the most common clinical finding encountered in clinical practice\(^2\). Pleural fluid analysis is necessary to ascertain the nature of effusion and to find out the etiology of it. Pleural effusion may be due to transudative or exudative causes. Transudates are secondary to remote (non pleural) pathology, causes may be Congestive Heart Failure, Nephrotic Syndrome, Hepatic Cirrhosis etc. An exudate indicates primary involvement of pleura and lung such as infection and demands immediate attention. Causes of exudates may be Tuberculosis, Bacterial Pneumonia, Pulmonary Abscess, Malignancy etc\(^3\).

Based on WHO data for 2007, all forms of TB in India accounted for almost 21% of the estimated TB burden in the world. The pulmonary tuberculosis is often associated with pleural effusion. It has been reported to occur in 2-38 % of children with pulmonary disease, but it is more likely to occur in adolescents and adults\(^4\).

The diagnosis of TB pleural effusion based on pleural tap which includes AFB staining and culture for AFB. AFB staining is positive only in 10 – 25 % of cases while culture for AFB is positive in less than 25 % of cases\(^5\). Even sensitive technique like PCR shows positive result only in 50 % of cases\(^6\). Thus a pleural biopsy has been considered the gold standard in diagnosis of TB pleural effusion but it is invasive\(^7\). The diagnosis in Tuberculous pleural effusion cannot be established in 10 – 20 % of cases with these methods even in the best conditions\(^8\). Delay in the diagnosis and in the treatment results in poor prognosis and sequelae in up to 25 % of cases\(^9\).

Adenosine deaminase (EC 3.5.4.4) activity was first noticed by Gyorgy and Rothler. ADA is an ectoenzyme that is found in surface of many Cells. The enzyme is widely distributed in human tissues and is found in lymphocytes, leukocytes, liver, intestinal mucosa, skeletal muscle, spleen, kidney and erythrocytes\(^10\). However the amount of enzyme differ widely among tissues. The highest ADA levels in human are found in Lymphoid tissues and the lowest in erythrocytes\(^11\). ADA is the enzyme of purine metabolism which catalyses the deamination of adenosine to inosine and ammonia\(^12,13\).

Human ADA consists of three isoenzymes ADA\(_1\), ADA\(_1 + CP\), ADA\(_2\), distinguished from each other by electrophoretic technique. ADA\(_1\) is a monomeric protein with a molecular mass of approximately 35KDa (gene assignment, chromosome 20). ADA\(_1 + CP\) is composed of two ADA\(_1\) molecules connected via a combining protein with a molecular mass of approximately 280 KDa (gene assignment, chromosome 2 & 6). ADA\(_2\) is coded by a separate gene locus of unknown chromosomal position\(^14\). ADA\(_1\) exists in all human tissues including lymphocytes, monocytes while ADA\(_2\) is found exclusively in monocytes\(^15\). Adenosine deaminase induces proliferation and differentiation of lymphocytes, specifically T lymphocytes. The T cells release ADA during the process of activation in the presence of live intracellular pathogens. Thus ADA has been looked upon as a marker
of Cell Mediated Immune response and specifically T cell activation. ADA levels were found to be elevated in the pleural fluid of patients with TB way back in 1978. ADA1 activity is more commonly associated with pyogenic bacterial infection of the pleural cavity contributing 70% of total ADA activity. ADA2 is the one raised in TB pleural effusion accounting for almost 88% of total ADA activity. However, there is no clear advantage of using the ADA2 over the total ADA activity in clinical practice. The total ADA activity assay is in fact preferred for its rapid turnover and low cost.

AIM AND OBJECTIVES: Aim of the study is to evaluate the ADA activity in patients with tuberculous pleural effusion and transudative pleural effusion and to investigate the diagnostic role in tuberculous pleurisy.

MATERIALS AND METHODS: This study was conducted at our medical college hospital. One hundred cases admitted in the medical ward on account of pleural effusion were selected. Asper Lights criteria cases are classified into exudate and transudate. The final diagnosis was based on standard diagnostic criteria. For TB pleural effusion, the following criteria was adapted to label as a case of Tuberculous effusion. 1. Bacteriological confirmation of presence of Mycobacterium tuberculosis. (direct smear or culture). 2. Radiological finding consistent with tuberculosis exclusion of other clinical consideration. 3. Clinical presentation consistent with tuberculosis. ADA was estimated by the method of Guisti and Glanti’s using semi autoanalyzer.

PRINCIPLE: Adenosine deaminase hydrolyses Adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of blue coloured indophenol complex formed is directly proportional to the amount of ADA in the sample and it is measured at 620 nm at 37°C in semiautoanalyzer. One unit of ADA is defined as the amount of enzyme required to release 1 mol of ammonia per minute from adenosine at standard conditions. Normal reference range of pleural fluid ADA is < 30U/L.
Other Parameters:
1. Pleural fluid glucose was estimated by Glucose Oxidase – Peroxidase method.
2. Pleural fluid and serum protein was estimated by Biuret method.
3. Pleural fluid and serum LDH was estimated by DGKC, method using semi-autoanalyzer.

STATISTICAL ANALYSIS
Results are shown as mean ± SD. Student’s t-Test was employed to determine statistical significance. P Value less than 0.05 was considered statistically significant.

| TABLE:1 |
| STUDENT t - TEST ANALYSIS OF PLEURAL FLUID ADA BETWEEN GROUP I AND GROUP II |
| SAMPLE | MEAN | S.D | STATISTICAL INFERENCE |
| GROUP I (n = 50) | 102.86 | 22.61 | |
| GROUP II (n=50) | 16.48 | 3.02 | P=0.0001<0.05 | SIGNIFICANT |

| TABLE:2 |
| STUDENT t - TEST ANALYSIS OF PLEURAL FLUID GLUCOSE BETWEEN GROUP I AND GROUP II |
| SAMPLE | MEAN | S.D | STATISTICAL INFERENCE |
| GROUP I (n = 50) | 49.43 | 7.75 | |
| GROUP II (n=50) | 94.96 | 16.54 | P=0.0001<0.05 | SIGNIFICANT |

| TABLE:3 |
| MEAN ± S.D OF LDH IN GROUP I AND II |
| SAMPLE | MEAN | S.D | STATISTICAL INFERENCE |
| GROUP I (n = 50) | 264.76 | 43.37 | P=0.0001<0.05 | SIGNIFICANT |
| GROUP II (n=50) | 94.54 | 14.24 | | |

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DISCUSSION:
The conventional diagnostic tools are incapable of pinpointing the cause of pleural effusion. So several biomarkers like ADA, Interferon-gamma cytokines and C reactive protein have been proposed as an alternative non invasive means of establishing tuberculous etiology in cases of exudative pleural effusion. In our study we measured pleural fluid ADA in tuberculous pleural effusion (group I) and compared the same with transudative pleural effusion (group II). The mean pleural fluid ADA level was significantly high in group I (102.86 ± 22.61) when compared to transudative pleural fluid (group II) (16.48 ± 3.02) and the p value is statistically significant. Adenosine Deaminase is increased in Tuberculous pleural effusion (TB) because of the stimulation of T – cell lymphocytes by mycobacterial antigens. The most widely accepted cut off level of ADA for the diagnosis of TPE is 40 units / l. Elevated levels of ADA in TPE have been noted by several authors. Mean ± SD of pleural fluid glucose in group I was 49.35± 7.75 while it was 94.96±16.54 in group II. The pleural fluid glucose level <60mg/dl is indicative of TB pleural effusion. The mean and standard deviation of protein in tuberculous group was 4.51 ± 0.43 while in transudative group it was 2.22 ± 0.43. Since TB pleural effusion is an exudate, pleural fluid protein is >3gm/dl. Mean ± SD of pleural fluid LDH in tuberculous group was 264.76 ± 43.37 while in transudative group it was 94.54 ± 14.24. The LDH level is an indicator of the degree of pleural inflammation. The higher the value more inflammed the pleural surface.

CONCLUSION:
This study showed that pleural fluid ADA levels were significantly high in tuberculous pleural effusion as compared to transudative effusion. Thus ADA may be useful in differentiating tuberculous etiology from transudative pleural effusion. Since it has high sensitivity and specificity indiagnosis of TPE, ADA estimation can be included routinely in the diagnostic work up of tuberculous pleural effusion in clinical practice

REFERENCES:

| TABLE 4: MEAN ± S.D OF TOTAL PROTEIN IN GROUP I AND II |
|---------------|----------------|----------------|----------------|
| SAMPLE        | MEAN           | S.D            | STATISTICAL INFERENC |
| GROUP I       | 4.51           | 0.43           | P=0.0001<0.05 SIGNIFICANT |
| (n = 50)      |                |                |                  |
| GROUP II      | 2.22           | 0.43           |                  |
| (n=50)        |                |                |                  |


