



A study of pleural fluid Adenosine Deaminase activity in tubercular and non-tubercular pleural effusion

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ABSTRACT

One of the most common complication of primary tuberculosis in India is exudative pleural effusion. Adenosine deaminase (ADA) is an enzyme produced by activated T- lymphocytes in response to the mycobacteria tuberculosis infection. ADA levels in pleural fluid will be comparatively higher than the serum levels suggesting the local synthesis of ADA by activated T-lymphocytes. Thus the estimation of ADA in tubercular pleural fluid helps in the diagnosis of tubercular pleural effusion. The objective of this study was to find out the role of ADA levels to differentiate tuberculous and non-tuberculous exudative pleural effusions of other aetiologies. The study comprises of 50 randomly selected cases, having of pleural effusion of different aetiologies and are admitted in the medical ward at a tertiary care hospital. The study found that pleural fluid ADA levels were significantly higher ($p < 0.001$) in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions. Hence, determination of ADA level in exudative pleural effusions is cost effective, rapid and simple procedure which should be considered for routine investigation of patients with pleural effusion, especially in a country like India where tuberculosis still overwhelmingly out numbers, the other causes of lymphocytic pleural effusion.

Keywords: Adenosine Deaminase, Exudative pleural effusion, Mycobacterium Tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a serious global health problem, as one-third of the world population is estimated to be infected with Mycobacterium tuberculosis and 8.7 million new active cases seen annually.¹ Tuberculosis is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent and 1.4 million died from TB annually.

In India due to low socioeconomic status, unawareness about hygienic practices has led to increased cases of TB. Pleural effusion is one of the most common causes of exudative pleural effusion and results as a complication of primary pulmonary tuberculosis which causes the rupture of tubercular cavity into the pleural space.

Apart from other causes of pleural effusion TB stands high in rate, although tubercular pleural effusion can resolve spontaneously but up to 65% untreated tubercular pleural effusion can develop active tuberculosis.²

So rapid and accurate diagnosis and prompt treatment is necessary for tubercular pleural effusion.

To differentiate tubercular pleural effusion from non-tubercular pleural effusions like malignancy, pneumonic effusions, transudative effusions etc, requires confirmatory test. A definite diagnosis of tuberculous pleural effusion can be made only when tuberculosis bacillus is found either in the smear or culture of pleural fluid material, pleural tissue or granuloma in the parietal pleura in pleural biopsy specimen.³ Tubercular bacilli are

rarely positive in pleural fluid smear or culture. Culture reports take 6 to 8 week time and are not widely available in all health care centres.

However, pleural biopsy is an invasive procedure and is associated with life threatening complications. There is need for rapid, safe simple, sensitive and non invasive laboratory test for the diagnosis of tubercular pleural effusion.

Adenosine deaminase is an enzyme of purine catabolism, it catalysis the conversion of Adenosine to Inosine. Its principles biologic activity is detected in T-lymphocytes and its level remains high in disorders where cellular immunity is stimulated and tuberculosis is one such disease. ADA level in tubercular pleural effusion is markedly increased compared to non-tubercular effusions. Hence this study was undertaken to determine ADA activity in pleural fluid and its role in differentiating the cause of pleural effusion.

MATERIALS AND METHODS

The study group comprised of 50 randomly selected cases of exudative pleural effusion with different aetiologies. Both male and female patients in the age group of 22 to 65 years admitted in the medical ward from a tertiary care Hospital, Adichunchanagiri Institute of Medical Sciences, Bellur.

Inclusion criteria

1. Clinically diagnosed cases of exudative pleural effusion of tubercular and other different aetiologies



(Based on the concentration of protein in pleural fluid >3gm/dl and cell count >1000cells/ μ L).

2. Cases with transudate pleural effusion

Exclusion criteria

History of typhoid fever, acute viral hepatitis and cirrhosis of liver were excluded.

The cases were confirmed and grouped based on clinical and X-ray results. With the informed consent of the patient, the site for pleural fluid aspiration was selected either in mid axillary line or posterior axillary line in the 6th, 7th or 8th intercostal space. Under aseptic precautions skin, subcutaneous tissue, intercostal muscles and parietal pleura are infiltrated with 5 to 10 ml of 2% lignocaine.

Thoracentesis was performed using 18 G needle attached to a 20 or 50 cc syringe at the proposed site and fluid is aspirated and collected into sterilized bottle.

The pleural fluid were analysed for protein, sugar, lactate dehydrogenase (LDH) and ADA levels.

Estimation of pleural fluid glucose level is done by glucose oxidase method, total protein by Biuret method, LDH by kinetic method in ERBA-CHEM-5 semi automated analyzer.

Adenosine deaminase was estimated by Giusti and Galanti colorimetric method. The ammonia which is produced during the reaction was assayed by modified Berthelot reaction.

Statistical analysis

The results were subjected to analysis using SPSS software and the results were expressed as mean \pm SD.

RESULTS

In the present study, we analyzed the ADA activity in 50 cases of pleural effusion with different causes, out of which 30 cases were of tubercular aetiology, 5 cases were malignant, 10 cases of para pneumonic and 5 cases were of transudative effusion as shown in the Table 1 and chart 1.

Table 1: Distribution of different types of pleural effusion with their aetiologies

Causes of pleural effusion	No. of cases	Percentage (%)
Tubercular	30	60
Malignant	05	10
Para pneumonic	10	20
Transudative	05	10

The study result showed the ADA activity in tubercular effusion was significantly higher (88.53 ± 17.7 , $p < 0.001$) compare to non – tubercular pleural effusion like malignant (34.6 ± 8.5), parapneumonic effusions (26.4 ± 9.3) and transudative effusion (13.8 ± 3.2). This indicates that, estimation of ADA is a very good parameter to

differentiate tubercular from non tubercular pleural effusion.

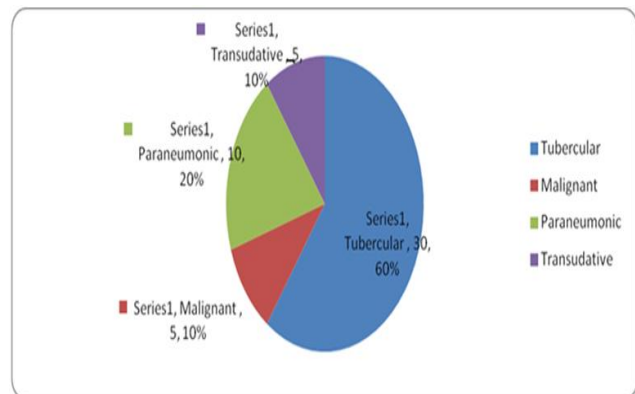


Chart 1: Distribution of different types of pleural effusion

DISCUSSION

In our study, all cases of exudative pleural effusion (tuberculosis, malignant and para pneumonic effusion) showed higher level of protein (>3.5gm/dl) and LDH (>200mg/dl) than the transudative pleural effusion cases except the glucose level. Which is in accordance with the Light's criteria of classifying transudative and exudative effusions.

The activity of ADA was found to be more in tubercular pleural effusion than in cases of non-tubercular pleural effusions and found to be statistically significant ($p < 0.001$).

Piras M.A., et al (1978) were one of the earlier workers who studied ADA activity in pleural effusion of different aetiology and concluded that the tubercular pleural fluid ADA activity was significantly higher when compared to different non tubercular cases.⁴

S.K. Sharma., et al., 2001 studied the ADA activity in pleural effusions of tubercular and non tubercular aetiology such as malignancy, para pneumonic, empyema, transudative, immunological disorders (like SLE, rheumatoid arthritis sarcoidosis etc.) and found that, ADA activity in tubercular pleural effusion was high when compared to non – tubercular pleural effusion.⁵ Our present study findings are consistent with the above workers.

In tuberculosis there are increased numbers of T-lymphocytes and macrophages in pleural fluid which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes.

Tom Petterson., et al, in 1984, studied ADA activity both in serum and pleural fluid in patient's pleural effusions of different aetiology. They found higher levels of ADA in tubercular pleural effusion and they also found higher ADA activity in pleural fluid than in serum indicating a local synthesis of ADA by T- lymphocytes.⁶

R. P. Sing, et al (1986), showed that, pleural fluid ADA activity was significantly higher in tubercular effusion, when compared to malignant and transudative effusions.⁷

Gilhothra R., et al., (1989), compared tubercular pleural fluid ADA with non – tubercular pleural fluid ADA and concluded that, higher values of ADA is strongly

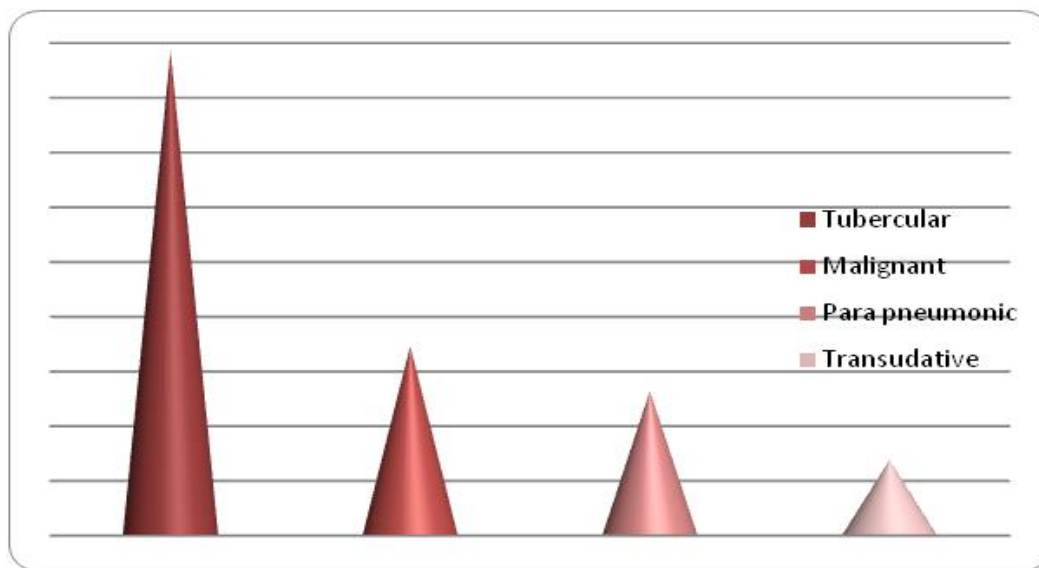
suggestive of tubercular aetiology. However, in this work, 4 out of 26 cases of malignant effusions also showed higher ADA levels, but they concluded that, pleural fluid ADA levels of less than 40 units/L excludes tubercular aetiology.⁸

Table 2: Distribution of pleural fluid glucose and protein in various types of pleural effusion

Type of pleural effusion	Pleural fluid glucose range mg/dl	Pleural fluid glucose mean ± SD	Pleural fluid Protein range gms/dl	Pleural fluid protein mean
Tubercular	50-100	67.3±14.12	3.5-6.4	4.63±0.6
Malignant	40-82	78.4±9.16	4.0-5.1	4.38±0.5
Para pneumonic	48-62	54.8±5.05	3.8-5.0	4.49±0.6
Transudative	92-138	107±16.27	1.3-2.5	1.98±0.4

Table 3: Distribution of LDH and ADA in different types of pleural effusion

Type of pleural effusion	Pleural fluid LDH U/L	Mean ± SD	Pleural fluid ADA U/L	Mean ± SD
Tubercular	220-1316	518.36±207	50-117	88.53±17.7
Malignant	620-920	778.4±116.7	20-45	34.6±8.50
Para pneumonic	382-560	460.2±53.5	13-47	26.4±9.3
Transudative	90-160	120±29	10-18	13.8±3.2



Graph 1: ADA activity in pleural fluid of different types of pleural effusions

Table 2, 3 and graph 1 showing the values of pleural fluid protein, LDH and ADA levels in various conditions.

ADA is present in most cells of the organism and is associated with the proliferation and differentiation of lymphocytes, especially T lymphocytes. The concentration of ADA in these lymphocytes is inversely proportional to their degrees of differentiation.⁵ ADA has gained increasing attention as a diagnostic tool, especially in countries where the prevalence of TB is high.⁹⁻¹³

In our study the ADA activity in malignant effusions was less compared to tubercular pleural effusions.

CONCLUSION

In the present study an attempt has been made to estimate the pleural fluid ADA activity in pleural effusion of different aetiology.

Most current laboratory tests like pleural tissue culture and histopathology are slow, invasive, comparatively less sensitive and their results depends on the availability of expert opinion. From the present study it is evident that, measurement of ADA in pleural fluid is a rapid, safe simple, sensitive and non invasive laboratory test for the diagnosis of tubercular pleural effusion.

ADA estimation being a simple colorimetric method, is imminently suitable for the rapid diagnosis of tubercular effusion in field setting.

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