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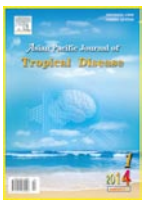

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Evaluation of usefulness of pleural fluid adenosine deaminase in diagnosing tuberculous pleural effusion from empyema

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PEER REVIEW

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Comments

The paper is a small retrospective study on the value of ADA in pleural fluid in the diagnosis of pleural tuberculosis. The authors findings support a cautious approach to ADA as diagnostic lab test which is in accordance with other reviews.
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ABSTRACT

Objective: To evaluate the utility of adenosine deaminase activity in the pleural fluid for the diagnosis of tuberculous pleural effusion from empyema of non-tubercular origin.
Method: A retrospective analysis of data was performed on patients who were diagnosed to have tuberculous pleural effusion and empyema of non tubercular origin. Among 46 patients at Kasturba Hospital, Manipal University, Manipal, Karnataka, India, from November 2012 to February 2013 who underwent pleural fluid adenosine deaminase estimation, 25 patients with tuberculous pleural effusion and 21 patients with empyema were diagnosed respectively. Adenosine deaminase in pleural fluid is estimated using colorimetric, Galanti and Guisti method.
Results: Pleural fluid Adenosine Deaminase levels among tuberculous pleural effusion(109.38±53.83), empyema (141.20±71.69) with P=0.27.
Conclusion: Pleural fluid adenosine deaminase alone cannot be used as a marker for the diagnosis of tuberculous pleural effusion.

KEYWORDS

Tuberculosis, Pleural effusion, Empyema, Adenosine deaminase

1. Introduction

Pulmonary tuberculosis (PTB) is a contagious bacterial infection which is the highly endemic disease in India that involves the lungs. It spreads to other organs. PTB is caused by the bacteria *Mycobacterium tuberculosis*[1]. Tuberculosis is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. Rapid diagnosis and prompt treatment is required to reverse the morbidity and mortality due to tuberculosis. Tuberculous pleural effusion is the commonest manifestation of extra-pulmonary tuberculosis (EPTB). Diagnosis of tuberculous pleural effusion is difficult because of non-specific clinical presentation and insufficient efficiency of traditional diagnostic methods due to paucity of bacteria in pleural cavity. Pleural biopsy is invasive and often requires several attempts to locate the infective loci but has been the gold standard method in diagnosis of tuberculous pleural effusion. Positivity for acid fast bacilli stain and histopathological study of pleura are very low and culture is time consuming. Enzyme linked immunosorbent assay, polymerase chain reaction, interferon assays are expensive tests for the identification of tubercle bacilli[2]. *Mycobacterium* is naturally resistant to variety of stresses due to their waxy thick wall. Inside the

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host, immune system senses the presence of the invading *Mycobacterium* and it stimulates the macrophage and T-cell accumulates at the site of infection. Macrophage becomes more efficient at killing foreign invader. The activated macrophage produce increased quantities of reactive oxygen intermediates. Oxidative stress plays a role in inflammation and inflammatory response increases the activity of adenosine deaminase activity (ADA)[3,4]. ADA is an enzyme involved in purine metabolism, catalyses the hydrolytic cleavage of adenosine and 2′deoxyadenosine, irreversible converting them into inosine and 2′deoxyinosine. Activity of ADA increases during cellular activation for energy demand to detoxify the toxic metabolites. It is ubiquitously distributed in human tissues. The physiological role of ADA is related to the proliferation and differentiation of T-lymphocytes. The level of ADA is ten times higher in lymphocytes than in erythrocytes, and in particular it is higher in T-lymphocytes than in B-lymphocytes with variation in differentiation. It′s a significant indicator of cellular mediated immunity[5-7]. ADA levels are estimated in various body fluids like pleural, pericardial, peritoneal fluids as well as in cerebrospinal fluid. Therefore ADA levels have been increasingly used in patients with tuberculosis and other manifestations of tuberculosis to diagnose the condition and reduce the mortality and morbidity due to PTB. ADA has been widely used as the most cost-effective pleural fluid marker for the diagnosis of tuberculous pleural effusion. The usefulness of ADA in differentiating the diagnosis between tuberculous pleural effusion and empyema is unclear. Therefore this study was aimed to record and compare the levels of pleural fluid ADA in patients with tuberculous pleural effusion and empyema of non-tubercular origin, and to evaluate the usefulness of this marker in the diagnosis of tuberculous pleural effusion from empyema of non-tubercular origin.

2. Materials and methods

2.1. Ethical statement

Ethical approval was obtained from institutional Ethics Committee Katurba Hospital, Manipal. IEC: 449/2012.

2.2. Subjects

Between November 2012 to February 2013, 46 individuals aged between 30-60 years were enrolled in the study. Patients were identified through tracing of pleural fluid specimens sent for adenosine deaminase level estimation to Clinical Biochemistry laboratory at Kasturba hospital, Manipal University, Manipal, Karnataka, India. Patients presenting with pleural effusion had pleural tap and subsequently pleural fluid analysis. Subjects with Empyema secondary to tuberculosis age more than 60 years and less than 30 years, were excluded from the study.

2.3. Classification of subjects presenting with pleural effusion

The diagnosis of tuberculous pleural effusion was confirmed if pleural fluid culture of *Mycobacterium tuberculosis* was positive or clinical features suggestive of tuberculosis like night sweats, chronic cough, fever, loss of weight, history of tuberculosis contact, past history of tuberculosis, radiological features suggestive of tuberculosis, subjects responding to anti-tubercular treatment.

The non-tuberculous pleural effusion group includes empyema patients diagnosed by gross appearance and microbiological examination for bacteria in the pleural fluid.

2.4. Sample

Aspirated pleural fluid samples were subjected to cytological, biochemical, microbiological examination and ADA activity. Hemolysed samples would give spuriously high ADA activity, hence these samples were discarded. The pleural fluid ADA level was determined by using colorimetric method of Giusti and Galanti.

2.5. Estimation of adenosine deaminase levels

To assess the adenosine deaminase levels in pleural fluid we adopted colorimetric, Galanti and Guisti protocol. ADA catalyses the deamination of adenosine leading to formation of inosine and ammonia. Ammonia forms intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution. Sodium nitroprusside is the catalyst. The ammonia concentration thus released, deamination by ADA is directly proportional to the examination of indophenol. The reaction catalysed by ADA is stopped at the end of incubation period by addition of phenol nitroprusside[8]. ADA levels were calculated and expressed in unit per liter (U/L). The following formula used for calculating adenosine deaminase levels:

$$\frac{\text{Test-Test Blank}}{\text{Reagent-Reagent Blank}} \times 250$$

Whereas, Normal level of pleural fluid ADA=10-40 U/L.

2.6. Statistical analysis

Data were compiled and statistical evaluations were performed using Statistical Package for the Social Sciences (SPSS) 16.0. Data are expressed as mean±standard deviation. Independent *t* test used wherever appropriate. *P* value<0.05 were considered to be statistically significant.

3. Results

Totally 46 pleural effusion samples successively obtained from the standard procedure of needle aspiration. Out of which 25 (males=16 and females=9) were diagnosed to be having tuberculous pleural effusion and 21 (males=14 and females=7) of them had empyema of non tubercular origin.

Mean and standard deviation of pleural fluid ADA in tuberculous pleural effusion was found to be 109.38±53.83 and in empyema of non tuberculous origin was found to be 141.20±71.69 (Table 1, Table 2). The correlation between the two groups did not show statistical significance between the pleural fluid ADA levels with *P* value being >0.05.

Table 1

The mean, standard deviation, standard error of mean of pleural fluid ADA between 2 groups.

Groups	*n*	Mean±SD	Mean±SEM
Tuberculous pleural effusion	25	109.38±53.83	109.38±10.76
Empyema	21	141.20±71.69	141.20±15.64

Table 2

95% confidence interval of pleural fluid ADA between 2 groups.

95% confidence interval	
Lower	Upper
−69.15	5.51

The ADA activities in the pleural fluid samples were increased in both the clinical conditions without any statistically significant difference in the mean and standard deviation between the two groups.

4. Discussion

In this study, there was no statistically significant difference(*P*>0.05) between pleural fluid ADA levels in patients of tuberculosis pleural effusion and those with empyema of non-tuberculous origin. Result of this study showed that ADA levels in the aspirated pleural fluids are of no considerable value in differentiating among tuberculous pleural effusion and empyema of non tuberculous origin. Possible cause for elevated ADA in empyema of non tuberculous origin is, the clearance capacity of lungs is decreased leading to increased number of cells in the pleural fluid and the recirculation of the activated lymphocytes may cause a high ADA activity.

Adenosine deaminase estimation in pleural fluid has long been taken as a marker for diagnosis of tuberculous pleural effusion. In the presence of live intracellular pathogen inside the host, activation of T-cell releases ADA. Thus ADA has been looked upon as a marker of cell mediated immune response and specifically T-cell activation. Isoforms of ADA arise from different gene loci, of which ADA 1 is found in all cells while ADA 2 is exclusively in monocytes. ADA 1 is raised in pyogenic bacterial infection contributing median of 70% total ADA activity and ADA 2 is raised in tuberculous pleural effusion accounting for almost 88% of total ADA activity. Although estimation of ADA isoforms are better than total ADA for diagnosing tuberculosis, the separation of ADA into its isoenzymes is more expensive than the total estimation of ADA, so far utility of the tests for isoforms of ADA are relatively limited[9].

Piras et al were first to report high ADA levels in tuberculous pleural effusion[10]. Subsequently several workers explored its efficacy in the diagnosis of tuberculosis and determined that the pleural fluid ADA level less than 40 U/L virtually exclude the diagnosis of tuberculosis[11].

In 2007, a systemic review of ADA by the NGS Health Technology Assessment Programme concluded that there is no evidence to support the use of ADA test for the diagnosis of PTB[12]. To conclude, ADA estimation is not useful in differentiating tuberculous pleural effusion from empyema of non tuberculous origin which goes with the above study. The limitation of our study being small sample size, further studies with larger groups may help us to obtain reliable results.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The diagnosis of EPTB is notoriously difficult and hampered by the paucity of bacteria in EPTB lesions. Especially in HIV co-infected patients, a presumptive diagnosis is often reached based on clinical case definitions only (WHO (2007): Improving the diagnosis and treatment of smear-negative pulmonary and EPTB among adults and adolescents: Recommendations for HIV-prevalent and resource-constrained settings). Simple laboratory tests to improve the diagnostic algorithms are urgently needed.

Research frontiers

Recent developments of simple, affordable and more widely available nucleic-acid technology in the diagnosis of tuberculosis have led to attempts to use this technology in the diagnosis of EPTB (Vadwai V. *et al* 2011: Xpert MTB/RIF: a new pillar in diagnosis of EPTB?

Related reports

ADA as a surrogate marker for tuberculosis associated effusions is interesting and would be helpful in the clinical setting. Individual recent reports have found it of diagnostic value (Sahn SA *et al*. 2013: Can tuberculous pleural effusions be diagnosed by pleural fluid analysis alone?), nevertheless other reviews have questioned its impact on the diagnostic algorithms for TB effusion.

Innovations & breakthroughs

This paper supports the feeling shared by many clinicians that ADA lacks sensitivity and specificity to be of significant help in the differential diagnosis of pleural effusion in tuberculosis.

Applications

Further research is needed in ADA and other laboratory tests need to facilitate the diagnosis of pleural and other forms of extra-pulmonary tuberculosis.

Peer review

The paper is a small retrospective study on the value of ADA in pleural fluid in the diagnosis of pleural tuberculosis. The authors findings support a cautious approach to ADA as diagnostic lab test which is in accordance with other reviews.

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