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Original Research Article

Adenosine Deaminase (ADA): A Biochemical Marker for Diagnosis of Tubercular Pleural Effusion

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ABSTRACT

Introduction: TB is a common cause of pleural effusion in countries like India where it is highly prevalent this study was designed to find out the role of ADA estimation in differentiation of TPE and non-TPE and to evaluate the diagnostic efficacy of ADA levels in TPE.
Materials and Methods: It was a hospital based study comprising of 73 patients, of either sex or age more than 20 years. They were divided into 2 groups: TPE and Non- TPE.
Results: Out of 73, 42 were TPE patients and 31 were Non-TPE patients. The mean age of 73 patients was 45.11 ± 15.71 years. Mean age of TPE patients was 43.69 ± 16.27 years and Non- TPE patients was 47.03 ±14.95 years. Mean ADA levels in all TPE patients was 68.362 ± 21.55 IU/L and in Non-TPE patients was 23.34 ± 7.54 IU/L, $p<0.0001$.The sensitivity and specificity of ADA was 100%.
Conclusion: ADA level of less than 40 IU/L virtually excludes the tubercular etiology in PE cases and thus may be useful in differentiating tuberculous etiology from other causes of PE. Although we have many methods for the diagnosis of TB, for example PCR, AFB staining and culture but these methods are costly, time consuming and do not provide enough sensitivity and specificity. Therefore, ADA estimation being a low cost, simple, rapid and non-invasive test should become an integral part of the diagnostic work up of PE in suspected cases of tuberculosis. As India has a high prevalence of TB and the sensitivity and specificity of this test is high in this population.

Key words: *Tuberculosis (TB), Pleural effusion (PE), Adenosine deaminase (ADA.)*

INTRODUCTION

The most common cause of pleural effusion (PE) worldwide (30-60%) is tuberculosis (TB). Tuberculous pleural effusion (TPE) accounts for 2 to 5% of all pleural effusions, approximately 1000 cases per year in the United States. [1] TB is a common cause of pleural effusion in countries like India where it is highly prevalent. [2] Successful diagnosis and treatment of TB has saved an anticipated 43 million lives between 2000-2014. TB remains one of the world's biggest threats, despite the advances made and despite the fact that nearly all cases can be cured. Worldwide, 9.6 million people are estimated to have fallen ill and 1.5 million people have died with TB in 2014. 58% of the 9.6 million new TB cases in 2014 were in The South- East Asia and in The Western Pacific Region. India accounts for 23 % of the global total and had the greatest number of cases. [3]

TPE is one of the most common forms of extrapulmonary TB. The immediate reason of the effusion is a

delayed hypersensitivity response to mycobacterial antigens in the pleural space. Because of this microbiological analyses are often negative and limited by the lengthy delay in obtaining results. [4] Although TPE can resolve spontaneously but up to 65% untreated TPE cases can develop active tuberculosis. So quick and accurate diagnosis and prompt treatment is necessary for TPE. Whenever a patient of PE presents, we usually investigate on the lines of gross, microscopic and biochemical parameters excluding Adenosine deaminase (ADA) level. Although lymphocytic predominant fluid is usually seen in TPE, but all lymphocytic predominant fluid can't be tubercular always. They can be malignant. So, it is necessary to differentiate among various causes of PE. Definitive diagnosis of TB is often difficult as in more than 50% of patients, pleura is the only site of infection. Finding can be negative as tuberculin test is non-specific. As, bacterial load is less; so pleural fluid culture for mycobacterium tuberculosis is also low (<20). [5]

Although we have many methods for the diagnosis of TB, for example polymerase chain reaction (PCR), Acid-fast bacilli (AFB) staining and culture but these methods do not provide enough sensitivity and specificity. [6] The organism mycobacterium tuberculosis is seldom detectable in pleural fluid, but the diagnosis of pleural tuberculosis has been greatly improved by the use of biochemical markers. [2]

ADA has been developed and extensively used for the diagnosis of TB. Many studies have confirmed the high sensitivity and specificity of ADA for initial diagnosis of extrapulmonary TB, such as tuberculous pleuritis, pericarditis and meningitis. [6] In areas with high TB prevalence, pleural fluid ADA levels greater than 40 U/L claim strongly for TB; in contrast, low levels of pleural fluid ADA have high negative predictive value in low-prevalence countries. [4,7] Pleural fluid ADA estimation is quick and comparatively inexpensive. [5]

ADA is an enzyme, which catalyzes the conversion ofadenosine to inosine by deamination and is also an essential enzyme of the purine catabolic pathway. ADA is present in all cell types; though, the amount of enzyme differs widely among tissues. The maximum ADA levels in humans are found in lymphoid tissues where it acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. [8] It also helps in maturation of monocytes transforming them to macrophage. ADA is an important indicator of active cellular immunity. It has been proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid. [9] The ADA levels increase in TB because of the stimulation of T cells by mycobacterial antigens. [6]

Helpfulness of ADA estimation in pleural fluid has been shown as a reliable chemical biomarker specially when there is suspicion of TB in endemic areas. Occasionally, the increase is marked in early stages of the disease and in some other conditions with neutrophillic effusions like in parapneumonic and empyema. [7] Researchers have established that ADA level rarely exceeds the cut-off set for TPE and non-tuberculous lymphocytic effusions. [10] Keeping in view the above facts, this study was designed to find out the role of ADA estimation in differentiation of TPE and non-TPE and to evaluate the diagnostic efficacy of ADA levels in TPE.

MATERIALS AND METHODS

This study was done in Department of Biochemistry, MMIMSR, Mullana, Ambala. It was a hospital based study comprising of 73 patients, of either sex or age more than 20 years, whose pleural fluid samples came in Biochemistry Lab for ADA levels. They were divided into 2 groups: TPE and Non- TPE as already diagnosed based on their history, sputum results, pleural fluid results and pleural biopsy.

ADA test was done in pleural fluid of all 73 patients by Enzymatic- Kinetic Method on semiautoanalyser. [11]

Normal range for ADA levels was: 10- 40 IU/L

Those patients, who had pleural fluid ADA levels more than 40 IU/L, were labeled as TPE and those with ADA levels less than 40 IU/L, were labeled as Non-TPE.

RESULTS

Out of 73, 42 were TPE patients and 31 were Non- TPE patients. The mean age of 73 patients was 45.11 ± 15.71years. Mean age of TPE patients was 43.69 ± 16.27 years and Non- TPE patients was 47.03 ±14.95 years. This difference was not statistically significant with p = 0.3728.

Mean ADA levels in all TPE patients was 68.362 ± 21.55 IU/L and in Non- TPE patients was 23.34 ± 7.54 IU/L. This difference was statistically extremely significant with p = 0.0001 [Table 1]

Table 1: Mean ± SD of Age and ADA levels in TPE and Non-TPE patients

	Mean ± SD		p value
	TPE	Non- TPE	
Age (years)	43.69 ± 16.27	47.03 ± 14.95	0.3728
ADA (IU/L)	68.362 ± 21.55	23.34 ± 7.54	0.0001 *
* Statistically extremely significant with p = 0.0001			

Range (min to max) of ADA in TPE patients was 40.18 to 122.3 IU/L and in Non- TPE patients was 10.64 to 35.2 IU/L.ADA levels in all 42 TPE patients were above the diagnostic cut off (40 IU/ L), while in Non-TPE, all were below the diagnostic cut off (40 IU/ L). To see the diagnostic efficacy of ADA, the sensitivity and specificity was calculated and both came out to be 100% in our study.

Out of the 73 patients, 31 were females; 16 TB and 15 Non- TB and 42 were males; 26 TB and 16 Non- TB [Figure 1]

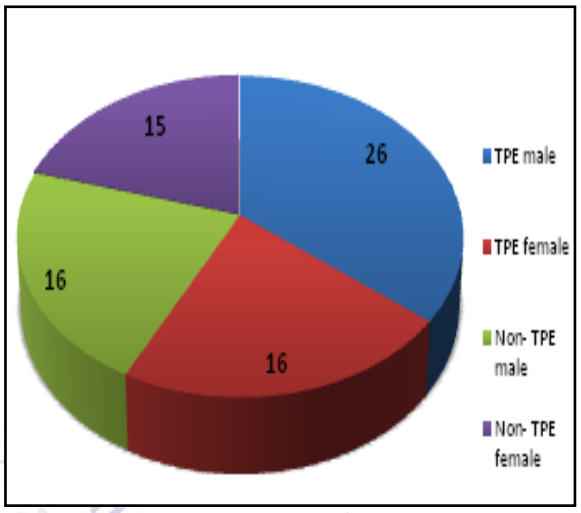

Figure 1: Sex- wise distribution of TPE and Non - TPE patients

Male: female ratio was 1.35:1. Mean ± SD of ADA levels in all TPE and Non-TPE males was 68.36 ± 21.55 and 21.125 ± 7.265 respectively. This difference was statistically extremely significant with p = 0.0001.

Mean ± SD of ADA levels in all TPE and Non-TPE females was 69.05 ± 19.82 and 24.600 ± 7.666 respectively. This difference was statistically extremely significant with p = 0.0001[Table 2]

Table 2: Table 1: Mean ± SD of ADA levels in TPE and Non- TPEMale and Female patients

		Mean ± SD		p value
		TPE	Non-TPE	
ADA levels (IU/L)	Males	68.36 ± 21.55	21.125 ± 7.265	0.0001 *
	Females	69.05 ± 19.82	24.600 ± 7.666	0.0001 *
* Statistically extremely significant with p = 0.0001				

DISCUSSION

TPE is a result of delayed hypersensitivity reaction to the tubercle bacilli. [7] Exudative Lymphocytic pleural effusions are commonly met in clinical practice but they frequently constitute difficult diagnostic problems. ADA is an enzyme in the purine salvage pathway which converts adenosine to inosine. Its levels are ten times higher in lymphocytes, particularly in T-lymphocytes than in erythrocytes. [2] The diagnosis based on pleural tap: AFB staining is positive in only

10 to 25% of the cases and culture for AFB is positive in less than 25% of the cases. [7]

In patients with exudative TPE, neutrophils predominate in the early stages of the disease, while an abundant mononuclear cell is a classical finding later. This is due to the proliferation and differentiation of lymphocytes, which release lymphokines, which in turn activate macrophages for an enhanced bactericidal activity. [12] Even pleural fluid cytology takes a back seat while inspecting the cause of an exudative PE, and is usually just evidence supporting our final diagnosis. [7]

Since the conventional diagnostic tools are incapable of locating the cause, so several bio-markers like ADA, interferon (IFN)-γ, C-reactive protein (CRP) and a variety of tumor markers and cytokines have been proposed as alternative non-invasive means of establishing tuberculous etiology in cases of exudative PE. [13]

In our study, ADA level cut off in TPE cases was taken as more than 40 IU/Land in cases with Non-TPE the ADA levels were found to be consistently below the cut-off. This is in agreement with many other studies like Bhushan et al, [2] Gupta et al. [7]

In our study, mean ADA levels in all TPE patients was 68.362 ± 21.55 IU/L and in Non- TPE patients was 23.34 ± 7.54 IU/L.ADA in the present study showed elevated level for tuberculosis as its activity is strongly related with an activation of T lymphocytes. Similar results have been found in study done by Gupta et al. [7]

This difference was statistically extremely significant with p = 0.0001. This proves that pleural fluid ADA levels can play a very significant role and can be utilized for differentiating TPE from those of Non- TPE as seen in other studies also. [7,14,15]

Range (min to max) of ADA in TPE patients was 40.18 to 122.3 IU/L and in Non- TPE patients was 10.64 to 35.2 IU/L. Our results were in concordance with studies of Verma et al [5] and Gupta et al. [7]

The sensitivity of the ADA as a serological marker for TPE depends on the prevalence of the disease in the population. In our study, the sensitivity and specificity both came out to be 100% each. India has a high prevalence of tuberculosis and so the sensitivity and specificity of this test will be high in this population.

CONCLUSION

ADA levels in Non- TPE do not exceed the cut-off set for tuberculous disease. The pleural fluid ADA levels were significantly higher in TPE when compared with Non- TPE. ADA level of less than 40 IU/L virtually excludes the tubercular etiology in PE cases and thus may be useful in differentiating tuberculous etiology from other causes of PE.

So, we can say that estimation of ADA level in pleural fluid is extremely helpful in establishing the etiology of TPE and to rule out other diagnosis especially of other diseases in which PE is seen such as malignancy and collagen vascular diseases (i.e. rheumatoid arthritis and systemic lupus erythematous).Therefore, ADA estimation being a low cost, simple, rapid and non-invasive test should become an integral part of the diagnostic work up of PE in suspected cases of tuberculosis.

India has a high prevalence of TB and the sensitivity and specificity of this test is high in this population. However, it is proposed that further studies might be conducted to verify more carefully the specificity and sensitivity of ADA and additional biochemical markers have to be developed and taken into consideration for assisting the differential diagnosis.

Limitation of study

Number of patients studied is small. So, conclusive criteria can't be established on this sample size. A large number of patients are required to confirm our findings further and establish the conclusive criteria.

The limitation of the ADA test is its inability to provide culture and drug sensitivity information, which is paramount

in countries with a high degree of resistance to anti-TB drugs.

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