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SERUM DEAMINASE ACTIVITY IN PULMONORY TUBERCULOSIS PATIENTS IN SOUTH INDIAN POPULATION

Biochemistry

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ABSTRACT

Background: TB has a long incubation period, with the timeline for transition from infection to expression lasting months or decades. Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes Mycobacterium tuberculosis. TB remains one of the major health problems in Bangladesh and worldwide.

Objectives: Our study aim is to investigate the diagnostic value of serum ADA level (as an alternative method) for diagnosis of PTB.

Methods and Materials: 3 ml of blood was taken from patients (40 nos) with Pulmonary Tuberculosis (PTB) and controls (40) under aseptic conditions and done the ADA assay in central laboratory of Biochemistry.ADA measured by Giusti and Galanti colometric method.

Result: Our results showed that mean serum ADA in pa¬tients with pulmonary TB is significantly higher than in the normal population.

Conclusion: The present study shows that a simple, inexpensive, highly sensitive and specific test like ADA estimation should be employed routinely to differentiate between tubercular and non-tubercular etiology in patients. ADA may be used for early diagnosis of TB, especially in case of negative AFB smear from the body specimens.

KEYWORDS

Tuberculosis, Adenosine Deaminase, National Tuberculosis Program.

INTRODUCTION:

Tuberculosis (TB) is a serious global health problem. Delay in diagnosis and in the start of effective treatment results in poor prognosis and sequalae in upto 25% of cases [1-2].Usefulness of adenosine deaminase (ADA) estimation in pleural fluid has been shown as a reliable chemical bio-marker specially when there is suspicion of tuberculosis in endemic areas. Sometimes the increase is marked in early stages of the disease and in some other conditions with neu-trophillic effusions like in parapneumonic and empyema [3]. Researchers have established that ADA level rarely exceeds the cut-off set for tuberculous effusion in non-tuberculous lymphocytic effusions[4].

Adenosine deaminase (ADA) is essential for the differentiation of lymphoid cells; in particular, T cells, and is found to play an important role in the maturation of monocytes to macrophages[5]. Also, ADA is considered to be an indicator of cell-mediated immunity [6].

Monocyte/macrophage activation by intracellular infection and inflammatory diseases leads to the release of ADA and elevated levels in serum. Increased serum ADA levels in pulmonary TB may result from a stimulation of cell-mediated immunity [7].

In patients with tuberculous exudative pleural effusion, neutrophils predominate in the early stages of the disease, while abundant mononuclear cells is a classical finding later and is believed to be due to the proliferation and differentiation of lymphocytes which release lymphokines, which in turn activate macrophages for an enhanced bactericidal activity [8,9]. Even pleural fluid cytology takes a back seat while investigating the cause of an exudative pleural effusion, and is usually just evidence supporting our final diagnosis.

Thus ADA can be used for ruling out suspected cases of tuberculosis and can be a very effective screening test. India has a high prevalence of tuberculosis and the sensitivity and specificity of this test will be high in this population. Therefore ADA estimation being a simple, low cost, rapid and non-invasive test should become an integral part of the diagnostic work up of exudative pleural effusions in suspected cases of tuberculosis.

Based on the background of the study, our study aim is to investigate the diagnostic value of serum ADA level (as an alternative method) for diagnosis of PTB.

MATERIAL AND METHODS:

40 patients with Pulmonary Tuberculosis (PTB) diagnosed based on the National Tuberculosis Program (NTP) with at least two positive sputum smears or a positive sputum smear plus clinical or radiographic evidence of PTB or a positive smear plus a positive sputum culture of mycobacterium tuberculosis. The second group included 40 controls, who where healthy in every aspect.

After obtaining the informed concerned and the completed questionnaires, 3 ml of blood was taken from each patients and controls under aseptic conditions and done the ADA assay in central laboratory of Biochemistry.ADA measured by Giusti and Galanti colometric method. Serum Adenosine Deaminase (ADA) activity was expressed in IU/L.

The ANOVA analysis was used to compare groups for quantitative variables and sensitivity analysis and Crow Rock chart were used to determine the sensitivity and specificity of Serum Adenosine Deaminase for PTB diagnosis. ROC (Receiver Operator Characteristic) curves were used to determine a cut off values for the ADA test. A test values below 4 was considered to be normal and above 4 to be abnormal. A non parametric method was used if they were not normal. All statistical analysis was performed by the SPSS-11.5 version.

RESULTS AND DISCUSSION:

TABLE – 1: Distribution of patients with positive results according to serum adenosine deaminase cut off points among studied group.

GRAPH -1: Sensitivity and Specificity serum adenosine deaminase activity in studied group.

TB is still responsible for the most human deaths caused by a single infectious agent [10]. Adenosine deaminase (ADA), an enzyme responsible for the conversion to inosine and deoxyinosine, which is involved in the proliferation and differentiation of lymphocytes, particularly, the T subtype [11]. It also plays a role in the differentiation of lymphoid cells and the maturation of monocytes to macrophages. The presence of ADA in pericardial and other body fluids reflects the

activity of the cellular immune response in the respective compartments, and in particular, the activation of T lymphocytes and macrophages. ADA has also been considered a marker of cell-mediated immunity [12]. Increased serum ADA activities were observed in many infectious diseases caused by microorganisms, which mainly infected macrophages. Since Mycobacterium tuberculosis infects lung macrophages, ADA may be released and detected in the serum of patients with TB. Mycobacteria can induce reactive oxygen species (ROS) production by activating phagocytes [13], and though these are important elements of the host's defense against mycobacteria, an enhanced ROS generation may promote tissue injury and inflammation. This may further contribute to immunosuppression, particularly, in those with impaired antioxidant capacity, such as HIV infected patients [14].

Despite all the progress in the diagnosis and treatment of TB, the disease is still a major health problem in many parts of the world, particularly in developing countries. Lack of timely TB diagnosis is the major cause of failure in controlling the disease. Although the standard diagnosis of PTB is based on *M. tuberculosis* isolation or direct observation of AFB in sputum examination yet, other diagnostic methods with shorter duration and acceptable sensitivity and specificity are essential. Rapid diagnosis of PTB from sputum by the Xpert MTB/RIF method using the polymerase chain reaction (PCR) is unavailable in low income countries due to the high costs of this method [15].

Total plasma ADA can be measured by a spectrophotometric method or colorimetric described by Guisti and Galanti in 1984.ADA 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. The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens [16].

Our results showed that mean serum ADA in patients with pulmonary TB is significantly higher than in the normal population (26 IU/L vs. 11 IU/L, $P < 0.001$). This finding is consistent with studies of Titarenko and colleagues in Russia [17], Agarwalm et al. in India [18], Agarwalm and colleagues found that serum levels of ADA in patients with pulmonary TB are significantly higher than in healthy subjects. Agarwal and colleagues in India also showed that serum levels of ADA in patients with sputum smear-negative pulmonary tuberculosis (culture positive) was significantly different from non-tuberculosis patients with other lung diseases such as lung cancer, pneumonia, pulmonary abscess and bronchiectasis. Bolursaz et al. believed that although, serum ADA level in pulmonary TB patients is higher than in normal individuals, ADA should not be considered as a suitable marker for differentiating between pulmonary TB and other pulmonary infections [19].

Our results showed that a serum ADA level of > or = 26 IU/L (cut off point) has a sensitivity of 35% and specificity of 91% in patients with PTB. When the results of all of our studied patients are compared with the results of patients from other studies, mean serum ADA activities are found to be significantly ($P < 0.05$) higher in the sera of patients with active PTB.

For the cut off value range of 15 to 55.5 IU/L for ADA in patients with PTB the sensitivity and specificity ranged from 12% to 100%, and 86% to 100%, respectively. Indeed increase of serum ADA activity cut off point is associated with increased specificity but not sensitivity (except a few studies on children) of the test for diagnosing the active disease of tuberculosis [20, 21]. Our finding related to validity of the serum ADA test is consistent with most previous studies with low sensitivity (range: 12% - 44%) and high (range: 96% - 100%) specificity yet, disagrees with studies with high (100%) sensitivity. Therefore, according to our results indicating low sensitivity for serum ADA level, this test is not a useful tool for TB diagnosis. Based on high specificity for serum ADA level, this test is a useful test to rule out TB in suspected cases with negative microbiological results. In cases where there is no possibility of using other methods such as sputum culture or polymerase chain technique and in limited source areas with limitation of laboratory staff expert in mycobacteriology, this test could help rule out TB. It is clinically supporting test for TB.

ADA acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It also acts in maturation of monocytes transforming them to macrophage. ADA is a significant indicator of active cellular immunity. It increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages. For example, deficiency in ADA in humans manifests primarily as severe lymphopenia and immunodeficiency. Tarhan and colleagues [22] suggested that serum ADA activities can be used for the diagnosis of tuberculosis as a supplementary laboratory test in combination with clinical and laboratory findings. Other causes of increase in ADA activity include bacterial infections, rheumatic disease and lymphoproliferative disorders. The article reviews of Gupta BK et al. ADA estimation as an effective diagnostic criterion for tuberculous and non-tuberculous disease in pleural, ascitic, synovial fluids and Cerebro Synovial Fluid (CSF) [23].

Adenosine deaminase higher levels were found in patients with rheumatoid arthritis and other autoimmune diseases, which agree with the findings of other authors [24]. Therefore conclusions based on one study should be interpreted with caution. Some other diseases such as typhoid fever, infectious mononucleosis, liver disease, sarcoidosis, leukemia, brucellosis and rheumatoid arthritis are expected to be associated with high serum levels of ADA. We have excluded these diseases based on the clinical presentation of PTB, not on the basis of specific diagnostic tests, therefore further controlled studies are recommended. This study showed that serum ADA with high specificity percentage might be a useful alternative test to rule out diagnosis of PTB.

Furthermore, malnutrition, which is commonly present in patients with TB, may contribute to their impaired antioxidant capacity. Direct (unconcentrated) sputum smears microscopy is the primary test for diagnosing pulmonary tuberculosis in developing countries. This method is quick and inexpensive, but has relatively low sensitivity. The in-vitro culture of mycobacterium tuberculosis bacilli is the golden standard, but is time consuming [25]. The polymerase chain reaction (PCR) assay has shown good sensitivity and specificity in several studies [26, 27], but it requires extreme precision, accuracy, sophisticated lab equipment, and skilled technicians. A rapid diagnostic test may be helpful for a diagnosis of pulmonary disease [28,29]. Minor differences may be due to accuracy of doing laboratory examination, racial or varied socio-economic status, sample size, site of infection (e.g. pleural), microbiological status (smear positive or smear negative), disease severity, adult versus children and cut off points. To achieve better and more definitive results, further controlled studies are necessary to determine the diagnostic value of ADA activities in patients with active pulmonary tuberculosis.

To definitively determine the reference values for serum adenosine deaminase and to fully assess its diagnostic significance require significantly larger sample. For now, the serum concentration of adenosine deaminase can be used as an indicative EPTB parameter, in the context of which its findings should be interpreted as part of a complete clinical presentation of disease.

This test has 100% sensitivity and 94.6% specificity for diagnosing tuberculous etiology with positive and negative predictive values of 95.5% and 100% respectively. The method of ADA estimation is easy, simple and doesn't require expensive equipment or elaborate laboratory arrangement except a simple colorimeter. It takes only 2 hours and it is also cheap. ADA is an essential enzyme of the purine catabolic pathway catalyzing the deamination reaction from adenosine to inosine that increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. There is sufficient data supporting yield of ADA in various body fluids for the diagnosis of TB.

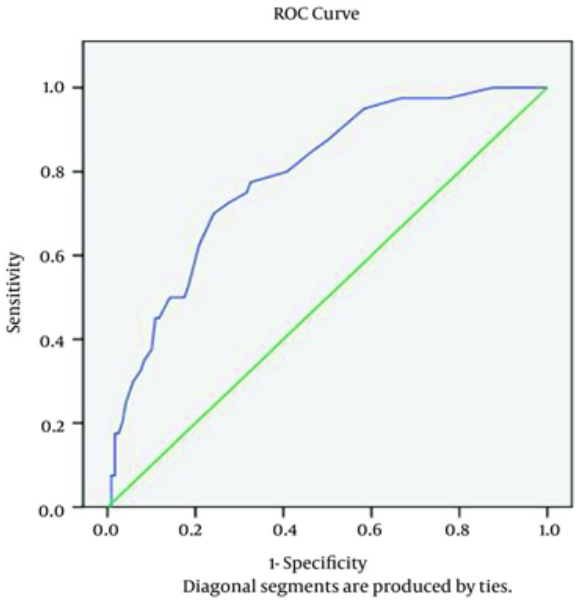
CONCLUSION:

In conclusion, although serum the ADA measurement is simple and inexpensive, it is not a useful test to differentiate PTB from other respiratory diseases, due to its low sensitivity and specificity. However, TAC may be a valid approach to determine the antioxidant status of TB and non-PTB patients. The present study shows that a simple, inexpensive, highly sensitive and specific test like ADA estimation should be employed routinely to differentiate between tubercular and non-tubercular etiology in patients.

ADA may be used for early diagnosis of TB, especially in case of negative AFB smear from the body specimens. However, culture is still the gold standard and mandatory for the confirmatory diagnosis. Prompt treatment of TB is crucial, especially in other countries like Bangladesh, where it is a high burden TB area. Further research regarding ADA is necessary to improve specificity, minimize false positive and choose the suitable cut-off value.

TABLE – 1: Distribution of patients with positive results according to serum adenosine deaminase cut off points among studied group

Groups	Cut off value						Mean IU/L	Range IU/L	P Value
	15.5 IU/L		19.5 IU/L		26 IU/L				
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**			
PTB	32 (80)	10 (25)	26 (65)	16 (40)	16 (40)	26 (65)	21	9-59	<0.001
Controls	0 (0)	40 (100)	0 (0)	40 (100)	0 (0)	40 (100)	11	7-15	<0.001



*Data represented in % Graph -1: Sensitivity and Specificity serum adenosine deaminase activity in studied group.

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