Original Article

Diagnostic value of ADA in Tuberculosis: a comparative study performed in Jammu, India

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Abstract

Background: Diagnosis of Tuberculosis in patients is usually made by clinical, radiological and laboratory investigations including the biochemical test of measuring serum Adenosine deaminase activity (ADA). Present study aims at finding usefulness of serum ADA in pulmonary tuberculosis.

Methods: The study was carried out on 100 patients suffering from pulmonary tuberculosis by taking history, physical examination and investigations including three consecutive (spot-early morning) sputum sample examination, Mantoux test, posteroanterior chest X-ray, ESR by westergren method and estimation of serum ADA activity. Comparative evaluation of these investigations was made and usefulness of detecting serum ADA was established in pulmonary tuberculosis.

Result: Out of 100 patients of pulmonary tuberculosis, serum ADA level was raised in 90 (90%) cases while Mantoux test was positive in 80 (80%) cases, chest X- ray was suggestive in 75 (75%) cases, ESR was raised in 70 (70%) cases and sputum AFB was positive in 64 (64%) cases.

Conclusion: Study of isoenzyme ADA is quite helpful in differentiating tuberculous and non-tuberculous etiology. Moreover being cost effective and less time consuming it should be done routinely as aid for diagnosis of tuberculosis.

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Introduction

Tuberculosis continues to be a major cause of morbidity and mortality worldwide. The diagnosis is usually based on clinical presentation, radiologic findings and positive tuberculin and/or BCG tests. However, clinic-radiological features are variable and the latter tests may be falsely negative. Under such circumstances, anti-tubercular therapy is started empirically. It therefore becomes imperative to find some rapid and useful tests for the diagnosis of tuberculosis. Adenosine deaminase (ADA) is involved in the propagation and differentiation of various lymphocytes, particularly T-lymphocytes, so that estimation of its level of activity in various body fluids has been used in the diagnosis of tuberculous effusions especially pleural forms [1]. Conversely, decrease in its activity has been noticed by Collazos et al. in treated cases [2]. Thora showed the raised activity of adenosine deaminase in the sera of new borns, six weeks after B.C.G. vaccination [3]. Mishra et al. noticed raised serum adenosine deaminase activity in a group of 51 tuberculous cases compared to 20 healthy individuals [4]. The same results were obtained by Ishii et al. in Japan [5].

Our study is mainly focused to find out the usefulness of serum ADA values in diagnosing cases of pulmonary tuberculosis.

Materials and Methods

The study was carried out on 100 patients suffering from pulmonary tuberculosis who attended in out patients department of Govt. medical college and hospital and Chest diseases hospital. Detailed clinical history, physical examination and investigations including three consecutive (spot early morning) sputum sample examination, Mantoux test, posteroanterior chest X-ray, ESR by westergren method and Serum ADA levels (MTB diagnostic kit; Tulip diagnostic (P) Ltd) were carried out on study group.

Pulmonary TB was defined in two categories:

1. **Sputum Smear Positive Pulmonary TB:**
   - A patient with at least two sputum specimen positive for acid fast Bacilli by microscopy.
   - A patient with at least one sputum specimen positive for acid fast Bacilli by microcopy, and radiographic abnormalities consistent with pulmonary TB.
   - A patient with at least one sputum smear positive for acid fast Bacilli by microscopy, which is culture positive for M. tuberculosis.
   - A decision by physician to treat with a full course of anti TB chemotherapy.

2**. Sputum Smear Negative Pulmonary TB**
   - Two sets (taken at least 2 weeks apart) of at least two sputum specimens negative for Acid Fast Bacilli and radiographic abnormalities consistent with pulmonary TB.
   - Lack of clinical response despite one week of a broad spectrum antibiotic administration and a decision by physician to treat with a full curative course of anti TB chemotherapy.

Inclusion criteria for Pulmonary TB

1. To have at least one positive sputum smear for acid fast bacilli.
2. Lack of any neoplastic or infectious disease (malignancy, rheumatologic disorder etc) according to physician's judgement and patient's medical documents.
3. To have abnormal chest X-ray that is reported by radiologist.
4. To have respiratory symptoms.

Exclusion criteria for pulmonary TB

1. No respiratory symptoms.
2. No previous contact with known tuberculosis according to patient's history.
3. Normal chest X-rays.

Result

Four groups of patients namely A (0-20 Yrs), B (21-40 yrs), C (41-60 Yrs) and D (> 61 Yrs) were formed, as shown in Table 1. The diagnosis of pulmonary tuberculosis was based on ESR, Sputum

AFB, Serum ADA, Mantoux test. Out of 100 patients of pulmonary TB 64 (64%) were sputum AFB positive and 36 (36%) were sputum AFB Negative. High serum ADA level (>33 U/L) was seen in 90 (90%) cases, 80 (80%) cases were Mantoux test positive (more than 10 mm erythema and induration) and 75 (75%) cases showed evidence of pulmonary tuberculosis on chest x-ray (Figure 1).

Discussion

ADA is an enzyme in the purine metabolic pathway. ADA activity detection is based on the principle that ADA 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 the blue colored indophenol complex formed is directly proportional to the amount of ADA present in the

Table 1. Showing different parameters with positive findings in the diagnosis of pulmonary tuberculosis.

AGE GROUP	High ESR	AFB positive	Mantoux test positive	High ADA	X-Ray Chest
A [0-20 yrs] N = 6	04	3	5	5	4
B [21-40 yrs] N = 16	13	12	12	13	13
C [41-60 yrs] N = 40	28	28	34	38	33
D [> 61 yrs] N = 38	25	21	29	34	25
Total	70	64	80	90	75

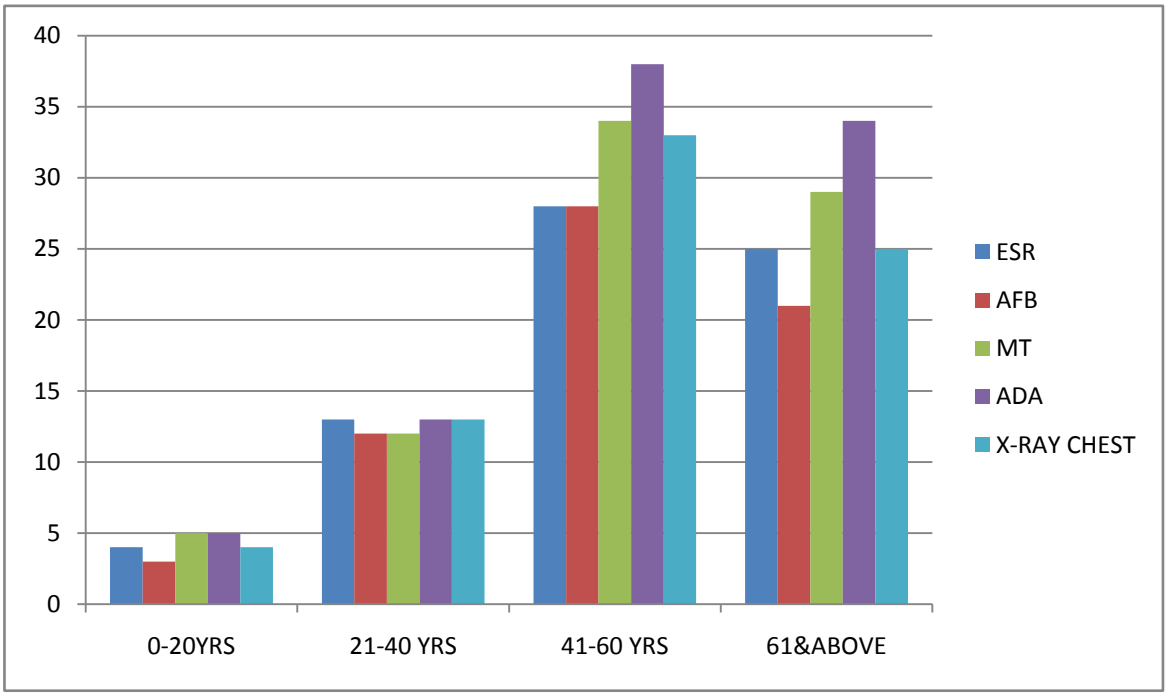

Figure 1: Graph showing positivity of different parameters in the diagnosis of pulmonary tuberculosis.

sample which is measured by spectrophotometery with filter at 570-630 nm.

ADA is shown to consist of two iso-enzymes (ADA1 and ADA2). The enzyme is scattered throughout the human body and its main physiological function is found in T-lymphocyte propagation and differentiation. The enzyme is higher in T-cells compared with B-cells with the ratio of 5-20 folds more [1]. ADA catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Two isoenzymes of ADA coded by different gene loci exist, namely ADA1 and ADA2, each with unique biochemical properties. The ADA1 isoenzyme is found as a monomer (ADA1m) and as a dimer (ADA1c), where two ADA1m molecules are combined with a combining protein. The ADA1 isoenzymes are found in all cells, with the highest activity in lymphocytes and monocytes, whereas ADA2 isoenzymes gene products appear to be found only in monocytes [1].

Although mycobacterial culture is sensitive and standard for diagnosing tuberculosis, the time for diagnosis requires a minimum of 2-3 weeks. Acid-fast bacilli smear, the rapid screening method for the diagnosis of pulmonary tuberculosis, is insensitive for detecting mycobacteria among tuberculosis patients [6].

The assay of Serum ADA activity in pleural and other infections is very useful in differential diagnosis, especially in the case of tuberculosis, which is characterized by significant increase in its activity [1]. However, the increased serum level of ADA has also been reported for viral and bacterial pneumonia, HIV infection, and extra pulmonary tuberculosis [7,8]. In fact diseases caused by intracellular micro-organisms are characterized by an elevated level of ADA in serum [7].

Ungerer et al. studied serum levels of ADA isoenzymes in 51 cases of confirmed tuberculosis (41pleural effusions, and 10 ascitic fluids), and 6 cases of bacterial pleural effusions (empyema), and noticed increased level of ADA2 in tuberculosis effusion (ADA2 / ADA total=88%), and ADA1 in non tuberculosis effusion (ADA1 / ADA total=70%), concluding that isoenzyme study is a helpful measure in differentiating these two kinds of effusions [1].

Thora et al. studied ADA levels of 100 newborn sera who were vaccinated with BCG showing a significant increase, indicating human cell-mediated immune response against mycobacterium antigens [3].

Mishra et al. evaluated serum ADA levels of 51 children with confirmed tuberculosis (pulmonary, peritoneal, meningeal, and bone), and 20 healthy controls showing significant increase in the first group with a p-value of <0.001 [4]. Collazos et al. performed a prospective follow up study of 25 cases of pulmonary and/or pleural tuberculosis with a normal immune response for a period of 6- months after initiation of treatment. There was a significant decline in the serum ADA values during the first two months in the patients as a whole (P=0.04), followed by stabilization of the serum ADA activity .This decline was due to a marked decrease in the serum ADA activity in 13 patients (52%) who had initial high levels of enzyme (p=0.03), whereas there were no changes in those patients with normal initial levels (p=0.27) [2]. Similar results were obtained by Ishii et al. in Japan together with a direct association between serum ADA level and erythrocyte sedimentation rate [5].

Conde et al. evaluated serum ADA in active pulmonary tuberculosis and other pulmonary infections and showed no significant difference between them [9].Their results were in disagreement with the report by Yasuhara et al. [10] in which serum ADA activity of children with active pulmonary tuberculosis was found to be significantly greater than those with bacterial or viral pneumonia.

In the present study, a high occurrence of pulmonary tuberculosis was found in the age group 41-60 yrs with serum ADA positivity of 95% (38/40) cases. Percent positivity of serum ADA was more, compared to other diagnostic tests like Mantoux test, sputum AFB, chest X ray and ESR.

Limitations

However elevated levels of ADA have been reported in effusions due to peritoneal, meningeal, pleural, pericardial involvement in several diseases like typhoid fever, infectious mononucleosis, bru-

cellosis and bronchogenic carcinoma involving stimulation of cell mediated immunity [11]. According to Giblett et al [12], a fully functionary cell mediated immune response is dependent on normal lymphocyte metabolism which is in part, regulated by the purine salvage enzyme, ADA. Therefore increased serum ADA activity is also found in other disease involving stimulation of cell mediated immunity.

Conclusion

As determination of ADA is not costly or time consuming, it should be done routinely, particularly if the diagnosis of tuberculosis is in doubt, and in sputum AFB negative pulmonary tuberculosis cases.

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Competing Interests

None declared.

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