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Original Research Article

A Comparative Study and Evaluation of Serum Adenosine Deaminase Activity in the Diagnosis of Pulmonary Tuberculosis

Rahul Jain[1], Rajendra Saugat[2], Gunjan Soni[3], Manak Gujrani[4], Jk Khatri[5], Ravi Chandak[6], Dheeraj Sharma[7], Tamanna Garg[8], Mohd Shakeel[9]

[1]Resident, Department of Respiratory Medicine, Sardar Patel Medical College Bikaner, Rajasthan, India

[2]Professor, Department of Respiratory Medicine, Sardar Patel Medical College Bikaner, Rajasthan, India

[3]Senior Professor, Department of Respiratory Medicine, Sardar Patel Medical College Bikaner, Rajasthan, India

[4]Senior Professor, Department of Respiratory Medicine, Sardar Patel Medical College Bikaner, Rajasthan, India

[5,6,7]Assistant Professor, Department of Respiratory Medicine, Sardar Patel Medical College Bikaner, Rajasthan, India

[8]Resident, OBG, Shri Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh, India

[9]Assistant Professor, Department of Biochemistry, Sardar Patel Medical College Bikaner, Rajasthan, India

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Corresponding author: Dr Mohd Shakeel
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Abstract

Background: A Comparative study and evaluation of serum adenosine deaminase activity in the diagnosis of pulmonary tuberculosis.

Methodology: This Hospital based cross-sectional study was conducted in the Department of Respiratory medicine, S.P. Medical College and P.B.M Hospital, Bikaner from Jan-2020 to Dec-2021 at Rajasthan after getting approval from ethical committee 100 cases and controls were randomly selected for the study. Written informed consent was obtained from each participant prior to participation in the study and the sample collection process. Data was collected and analysed with the help of Microsoft excel and statistical software SPSS and appropriate statistical tests were applied.

Results: In our study mean ADA level in cases (23.66±4.72 U/L) was significantly higher as compared to control (12.62±2.11 U/L). ADA level was significantly lower after 2-month treatment (15.22±3.18 U/L) as compared to ADA level at the time of diagnosis ((23.66±4.72 U/L). our study found diagnostic accuracy of ADA was 82.00%.

Conclusion: our study concludes in that serum ADA levels may be used as a supplementary aid for diagnosis of pulmonary tuberculosis and to evaluate the response to treatment at follow up.

Keyword: Pulmonary Tuberculosis, Adenosine Deaminase, Diagnostic Accuracy

Introduction

Tuberculosis is a bacterial disease spread from one person to another principally by airborne transmission. The causal agent is Mycobacterium tuberculosis (the tubercle bacillus). Tuberculosis can affect any organ in the body. Pulmonary tuberculosis is the most frequent site of involvement; extrapulmonary tuberculosis is less frequent. Only pulmonary tuberculosis is infectious [1].

India has been engaged in Tuberculosis (TB control activities for more than 50 years). Yet TB continues to be India's severest health crisis. India is the highest TB burden country in the world having an estimated incidence of 26.9 lakh cases in 2019 (WHO).

In 2020, after the intial 2 months of pandemic (March and April) TB notifications decreased by 38.00% as compared to January and February. Similar to trends in the previous years, over half of the total notifications are contributed by the five states namely Uttar Pradesh (20.30%), Maharashtra (8.84%), Madhya Pradesh (7.62%), Rajasthan (7.61%) and Gujarat (6.68%) [2].

Latent TB occurs when a person has the TB bacteria within their body, but the bacteria are present in very small numbers. They are kept under control by the body's immune system and do not cause any symptoms. Latent TB is one of the two types of TB. The other type is TB disease. This is sometimes known as active TB. People with latent TB does not feel sick and are not infectious. They cannot pass the TB bacteria on to other people. In addition they will usually have a normal chest x-ray and a negative sputum test. It is often only known that someone has latent TB because they have had a TB test, such as the TB skin test. An estimated 354 million people are latently infected with tuberculosis in India and form the next generation of future [3].

TB contact investigations are rarely and inconsistently carried out in resource-limited settings. In most low- and middle-income countries, it is included in the national policy to control and prevent TB; however, in the vast majority of countries, it is either not undertaken or is implemented on the basis of no or poor standards, because of the absence of clear definitions of index cases, contacts and procedures. Furthermore, the health personnel who should be involved are usually not clearly identified [3].

According to the WHO, all TB patients should be monitored during anti-tuberculosis treatment to assess their response to therapy. The monitoring basically concerns body weight and sputum smear examination which should be done, among others, at the end of the intensive phase of treatment. Despite the low positive predictive value of sputum smear examination during treatment, it has been documented to be well correlated to smear culture and the proportion of smear positive patients with sputum smear conversion at the end of the intensive phase is an indicator of TB program performance. Due to limited resource settings sputum culture is limited to only MDR-TB Patients and category 2 previously treated patients [4].

The National Tuberculosis Program was initiated in India during 1962 which was revised in 1997 as the Revised National Tuberculosis Control Program (RNTCP) that used WHO recommended (directly observed treatment, short-course [DOTS] chemotherapy) strategy. Intermittent, directly observed treatment, short-course (DOTS) was administered to tuberculosis patients nationwide, as per the guidelines of Revised National Tuberculosis Control Program (RNTCP). A DOTS strategy is recommended as the key to successful treatment outcomes for tuberculosis patients. Although DOTS was implemented throughout the country by the year 2006, the number of cases of TB continued to rise simultaneously with

increased number of relapses and drug-resistant TB cases [5].

In this regard, in March 2016, RNTCP introduced daily DOTS regimen with fixed-dose combination in a phased manner for the treatment of TB patients; to make the treatment more compliant, effective, reduce the relapses, and the incidence of drug resistance in TB patients. In Jaipur district the daily fixed dose regimen was started from NOVEMBER 2017 [6].

The Indian TB National Strategic Plan (NSP) 2017 - 2025 is the plan produced by the government of India (GoI) which set out what the government believed was needed to eliminate TB in India. The NSP 2017 - 2025 described the activities and interventions that the GoI believed would bring about significant change in the incidence, prevalence and mortality from TB. The state of Uttar Pradesh has the highest number of TB patients notified by both the public and private sector [1].

Adenosine deaminase (ADA) is an enzyme which plays a role in purine catabolism which catalyzes the conversion of adenosine to inosine and deoxyadenosine to deoxyinosine. The examination of ADA activity is fast, affordable, and has high sensitivity and specificity to be used as a supporting diagnosis of pulmonary TB (PTB). Elevated serum ADA levels in PTB patients conform to the severity of the disease, high bacterial isolation, and lung tissue damage. This suggests that ADA measurement is an additional criterion in assessing the response of TB treatment and is used as a prognostic tool in PTB patients [7]. Studies of serum ADA levels comparison in TB patients during TB treatment in Rajasthan (India) are limited.

Therefore, this study was designed to analyze changes in serum ADA levels in new PTB cases with smear positive tuberculosis that experienced sputum conversion, so as to assess its utility as a possible treatment monitoring method in PTB [8].

Materials and Methods

Study design: Hospital based cross-sectional study.

Study duration: From the approval of thesis till 31 december 2021 or till achievement of the sample size, whichever is earlier

Study place: Department of Respiratory medicine, S.P. Medical College and P.B.M Hospital, Bikaner

Sample size: A sample size calculation (Power of study=80.00% and Sample Size Calculation by MEDCALC 16.4 version software) showed that 100 patients were required, based on study conducted **by** K. Srinivasa Rao *et al*.

Power of study =80%

Allowable error=5%

100 cases of newly diagnosed pulmonary TB were included for the present study.

Sampling Method: Random sampling

Study Group: A total of 100 laboratory diagnosed pulmonary tuberculosis patients, who have consented to participate in the study during treatment was selected.

Control Group: 100 normal healthy age and sex matched individuals was selected as controls after getting their consent for participation.

Inclusion criteria for pulmonary TB

1. Cases diagnosed as a “new case” of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli with written consent.
2. Newly diagnosed pulmonary tuberculosis cases, who were willing to participate during treatment was included in the study group.

Exclusion criteria for pulmonary TB

1. Patients with extra pulmonary TB
2. Previously treated pulmonary TB patients.

Data Collection

This study was undertaken with the approval of the Institutional Ethics Committee of S.P. Medical College and PBM Hospital, Bikaner, and written informed consent was obtained from each participant prior to participation in the study and the sample collection process.

All selected cases under study group was analyze for complete blood count, ESR and serum ADA and control group were analyzed for serum ADA levels. Patients was treated with standard anti tuberculosis treatment as per RNTCP (DOT) regimen under the supervision of I/C of Respiratory Medicine dept.

Venous blood was drawn aseptically and collected in two sterile containers, one anticoagulated container and another plain container. The anticoagulated blood was used for total leukocyte count, lymphocyte count and ESR. Serum sample was collected from plain blood after centrifugation and used for ADA estimation.

ESR by Westergren's method [5], Plus semi auto analyzer. Leukocyte total count and differential count were measured in a semi-automated cell counter ABX Micros 60.

ADA estimation was done by standard colorimetric method as described by Guisti G Galanti enzymatic analyses. Adenosine deaminase activity was determined in serum by using ADA MTB diagnostic kit from Microxpress a division of Tulip diagnostics (P) Ltd [9,10].

Data Analysis

To collect required information from eligible patients a pre-structured pre-tested proforma was used. For data analysis Microsoft excel and statistical software SPSS was used and data was analyzed with the help of frequencies, figures, proportions, measures of central tendency, appropriate statistical test.

Results

Table 1: ADA level status wise distribution of study subjects

ADA at time of diagnosis	TB patients	Control	p-value
Mean	23.66	12.62	0.001
SD	4.72	2.11	

In our study mean ADA level in cases (23.66±4.72 U/L) was significantly higher as compare to control (12.62±2.11 U/L).

Table 2: ADA level in TB patients

ADA	At time of diagnosis	After 2 month	p-value
Mean	23.66	15.22	0.01
SD	4.72	3.18	

In our study, ADA level was significantly lower after 2 month treatment (15.22±3.18 U/L) as compare to ADA level at the time of diagnosis ((23.66±4.72 U/L)

Table 3: ADA diagnostic value

Cut off value of ADA level	22.39
Area under curve	0.570
p-value	0.01

In our study, cut off value of ADA level was 22.39 U/L and area under curve was 0.570.

Table 4: ADA diagnostic value

Sensitivity	72.00%
Specificity	88.00%

The best cut-off point was 22.39 U/L in which sensitivity and specificity were 72.00% and 88.00%, respectively.

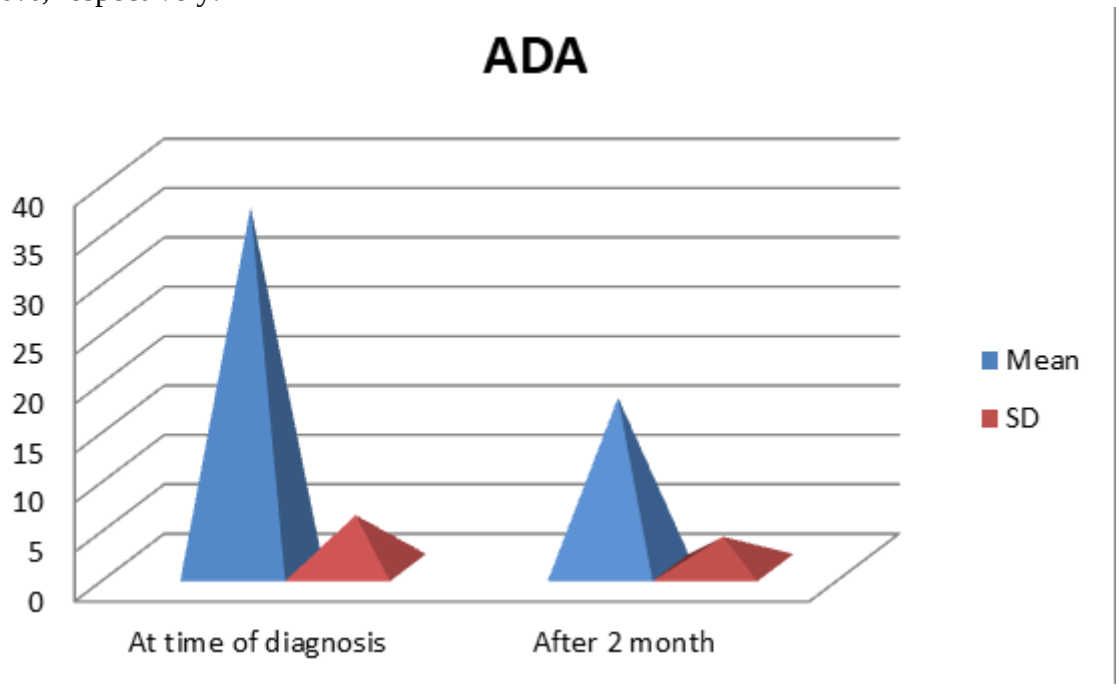

Figure 1: ADA level in TB patients

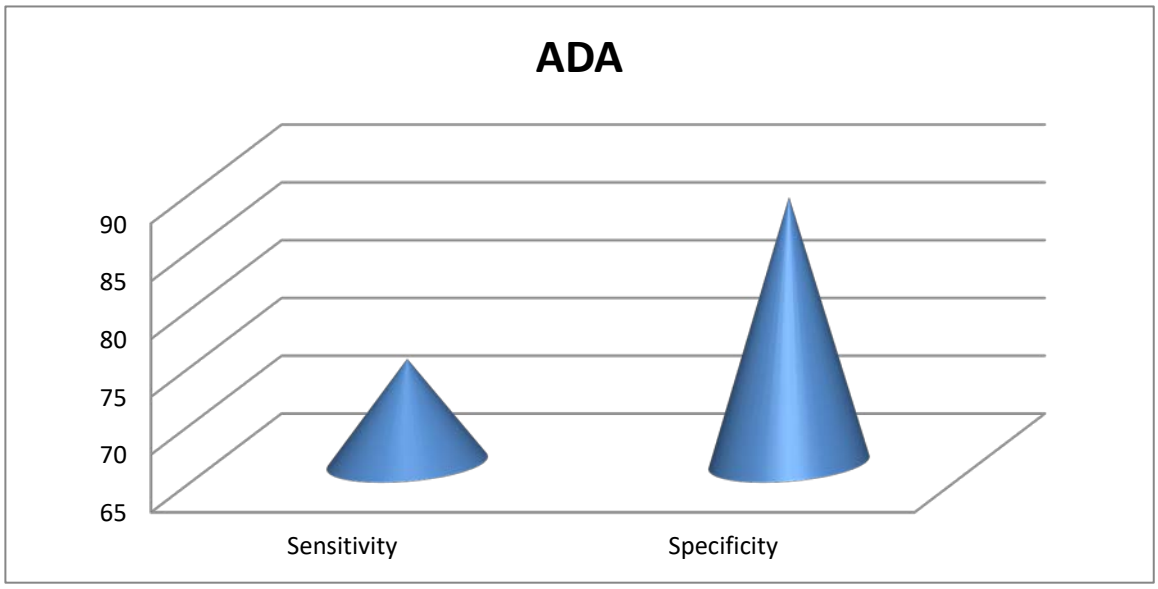

Figure 2: ADA diagnostic value

Table 5: ADA diagnostic accuracy

ADA level	Diagnostic accuracy
After cut off value 22.39 U/L	82.00%

In our study, diagnostic accuracy of ADA was 82.00%.

Discussion

Hospital based cross-sectional study conducted at department of Respiratory medicine, S.P. Medical College and P.B.M Hospital, Bikaner. A total of 100 laboratory diagnosed pulmonary tuberculosis patients, who have consented to participate in the study during treatment was selected. 100 normal healthy age and

sex matched individuals were selected as controls after getting their consent for participation. Cases diagnosed as a "new case" of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli. Only those newly microbiologically diagnosed pulmonary tuberculosis cases, who were willing to participate during treatment were included in the study group.

In our study mean age of cases was 44.06±10.02Yrs and control was 44.93±8.18 Yrs. Both groups were comparable.

Similar result was found by Shokrollah Salmanzadeh *et al* [11] was found that total of 160 subjects were included in this study. The PTB group consisted of 27 (67.5%) males and 13 (32.5%) females with a mean age of 49 years.

Similar result was found by Soedarsono Soedarsono *et al* [12] there were 26 TB patients in this study with an average age of 42.85 years.

Similar result was found by Shahla Afrasiabian *et al* [13] they found that most common age group for TB patients was 50s and for control group, it was 40s. Age average was 59 (±13.5) in TB patients and it was 49 (±15.6) for non-TB patients.

In our study, Male patients in cases group were 89.00% and in control group were 81 .00%. Both groups were comparable. PTB is common in men, and this is due to the greater smoking habits in men increased risk of incident TB and the high mobility causes the high transmission [14].

Similar result was found by Shokrollah Salmanzadeh *et al* [11] they found that total of 160 subjects were included in this study. The PTB group consisted of 27 (67.5%) males and 13 (32.5%) females.

Similar result was found by Soedarsono Soedarsono *et al* [12] PTB is common in men from 21/26 (80.8%) men to 5/26 (19.2%) women.

Shahla Afrasiabian *et al[13]* was found that from all 40 TB patients who participated in this study, 16 were males and 24 were females. From 42 participants in control group, 22 were males and 20 were females.

Tuberculosis is a major health problem in India and out of all its forms pulmonary TB is the commonest. A definitive diagnosis of pulmonary tuberculosis is made microbiologically. Chest radiography helps radiologically in predicting the disease. Serum ADA has shown some significant results in various studies, so the study was planned to find out the role of Serum ADA in Pulmonary TB. Previous numerous studies have shown increased ADA levels in body fluids like in tubercular pleural effusion, tubercular ascites, however the literature on the levels of Serum ADA is limited.

In our study mean ADA level in cases (23.66±4.72 U/L) was significantly higher as compare to control (12.62±2.11 U/L).Similar result was found by Soedarsono Soedarsono *et al* [12] they found Serum ADA level at the beginning of TB treatment was 26.40±9.619 and in healthy control was 10.18±2.39 while after the intensive phase treatment it was 19.67±8.118

Previous studies have reported that the diagnostic value of serum ADA for pulmonary tuberculosis is associated with controversial results. In studies of Al-shammary from Saudi Arabia [15], Afrasiabian and colleagues from Iran [13] and Dilmac and colleagues from Turkey [16] serum levels of ADA in patients with pulmonary tuberculosis were significantly higher than in other patients with lung cancer and bacterial pneumonia. Agarwalm 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*. [17] 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.

In our study, cut off value of ADA level was 22.39 U/L and area under curve was 0.570. The cut-off point was 24.31 U/L in which sensitivity and specificity were 72.00% and 88.00%, respectively.

This finding is consistent with studies of Shokrollah Salmanzadeh et al [11] they found that mean serum ADA in patients with pulmonary TB is significantly higher than in the normal population (26 IU/L vs. 10.7 IU/L, P < 0.001). Titarenkov and colleagues in Russia [18], Agarwal *et al*. in India [19], and Afrasiabi and colleagues in Iran [13] Agarwal and colleagues found that serum levels of ADA in patients with pulmonary TB are significantly higher than in healthy subjects [15]. Afrasiabi and colleagues in their study measured serum ADA levels in two groups of patients; cases with pulmonary tuberculosis and patients with non-tuberculosis infections (patients referred to the hospital for surgery) [13]. They found that serum levels of ADA in patients with pulmonary TB were significantly higher than in non-tuberculosis subjects. In some studies, including a review study by Dinnes *et al*. significant differences in serum ADA levels, between patients with TB and other patients with non-tuberculosis infection, have not been demonstrated [20].

In our study, ADA level was significantly lower after 2 month treatment (15.22±3.18 U/L) as compare to ADA level at the time of diagnosis (23.66±4.72 U/L)

Similar to our study Soedarsono Soedarsono *et al* [11], they found the mean serum ADA levels in PTB patients before receiving TB treatment was 26.40 IU/L and decreased during PTB treatment with serum ADA levels of 19.67 IU/L. Paired *t*-test showed a significant difference in serum ADA levels before TB treatment and after the end of the intensive phase of TB treatment with $P < 0.001$. These data suggests that ADA measurement is an additional marker in assessing therapy response or treatment monitoring in PTB patients. Rao *et al*. [21] reported the decrease in serum ADA levels during PTB treatment. Serum ADA levels showed a significant difference between serum ADA levels before and after the 2nd month of TB treatment with $P < 0.001$ (41.48 U/L vs. 29.66 U/L) [21-22]. The measurement of serum ADA could help the evaluation of therapy response. Other study reported the same results that serum ADA levels decreased during TB treatment

Changes of serum ADA levels can be used as a therapeutic response marker. Serial examination of serum ADA levels can be used to monitor therapy response of TB treatment, especially in patients with negative sputum microscopic examination results. The future study was needed by measuring the serum ADA levels at the baseline before therapy and followed up till the end of TB treatment [23].

Conclusion

We concluded that serum ADA levels may be used as a supplementary aid for diagnosis of pulmonary tuberculosis and to evaluate the response to treatment at follow up.

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