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ORIGINAL ARTICLE

# ADA Levels in Body Fluids as the Preferred Test to Rule Out Tuberculosis in Limited-resource Settings: Data from a Tertiary Care Hospital in Northern India

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ABSTRACT

In clinical practice, the diagnosis of tuberculosis (TB) continues to be a challenge. The goal of this study was to evaluate the reliability and impact of adenosine deaminase (ADA) enzyme testing as a biochemical marker in the continued management of suspected tuberculosis in a limited resource setting hospital. The retrospective data were collected from 2018 to 2021 and comprised the results of all ADA test assays done in the laboratory. All types of body fluids received for ADA testing were analyzed. Over the course of two years, 1461 samples for ADA assay testing were received. The average age of the study population was 56.69±11.7 years, with males accounting for the majority of the subjects (55.72%). Pleural fluid (N=817, 55.92%) was the most common type of sample received for the ADA assay. 114 (13.95%) of the 817 pleural fluid samples were found to be positive. A survey was conducted to obtain physician's response regarding reliability on ADA testing. 100% of them reported the supportive role of ADA levels in the workup of patients with suspected tuberculosis. In a limited resource setting, the ADA test, in conjunction with clinical and other laboratory findings, can help physicians to initiate early treatment in hospitals for the benefit of patients.



## INTRODUCTION

Tuberculosis (TB) continues to be a major public health issue, particularly in developing nations such as India. India is a TB-endemic country, and each year, a considerable number of cases of pleuritis, peritonitis, and meningitis are reported. However, regular monitoring, diagnosis, and treatment will be critical in eradicating this terrible disease. According to the World Health Organization (WHO) TB latest available statistics for India in 2019, there are an estimated 2.64 million active cases [1]. The Indian government declared in 2020 that it will eradicate tuberculosis from the country by 2025 through its national TB elimination program. The Indian's smallest Union Territory of Lakshadweep and one district in Jammu & Kashmir, Budgam, were the first to be certified TB-free [2].

There are several conventional procedures for its diagnosis, such as culture, but it takes around six weeks, which delays therapy commencement [3]. The culturing of the biopsy material from laparoscopy also necessitates an invasive process with numerous risks. Similarly, acid-fast bacilli smear (AFB) is utilised, but it has much lower sensitivity. Although advanced immunological approaches such as cellular interferon-release assays

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(IGRAS), antibody in lymphocyte supernatant (ALS) tests, and antigen-antibody detection have been launched, their function in disease diagnosis remains unclear [4]. In terms of polymerase chain reaction (PCR), this molecular technology aids in the diagnosis of *Mycobacterium tuberculosis* (MTB) even when the bacilli concentration is low, but the high cost of the PCR test limits its routine prescription by our physicians in low-resource settings. Another biochemical biomarker, adenosine deaminase (ADA) enzyme levels in different body fluids, is frequently administered to patients with high clinical suspicion of TB to expedite treatment. Because of T cell activation by mycobacterial antigens, the level of ADA rises in tuberculosis [5]. In humans, ADA deficiency primarily causes severe lymphopenia and immunodeficiency symptoms [6]. ADA is an important enzyme of purine catabolism. T- lymphocytes have the most quantity of ADA enzyme. Among the soluble immunoregulatory proteins of human plasma are adenosine deaminases ADA-1 and ADA-2. They are encoded by the ADA gene on chromosome 20q13.12 (OMIM 608958 or Entrez Gene ID 100) produced by all cells [7]. ADA is a biomarker of active cellular immunity. ADA-1 is found in RBC and many tissues. ADA-2 is found only in macrophages and monocytes [8]. It accumulates in body fluids during infectious illness induced by microorganisms invading macrophages. ADA-1 has external roles in addition to its intracellular functions, including the creation of a ternary complex with CD26 and A2a receptors that bridges two distinct cells as a co-stimulatory protein that influences T-cell proliferation [9]. It also aids in the development of monocytes into macrophages. ADA-1 transforms adenosine, an endogenous purine metabolite that suppresses pro-inflammatory and Th1-polarizing responses via leukocyte purine receptors, to inosine, an immunologically inactive compound [10]. Through adenosine receptors, ADA-1 improves T-helper 2 (Th2) immunity [11].

The ADA test is still regarded as a quick and cost-effective approach that may be performed even in basic care settings in underdeveloped nations. Furthermore, numerous studies suggest that it can be used to make an early diagnosis of extrapulmonary tuberculosis, such as tuberculosis pleuritis, pericarditis, and meningitis [12].

In this study, we aimed to evaluate the data from our hospital record to find out the prevalence, frequency of ADA testing in detecting suspected *M. tuberculosis* infections in different body fluids. The reliability and effectiveness of the ADA assay will be assessed via a questionnaire put forward to physicians across various specialties.

## MATERIALS AND METHODS

### 1. Participants

This study was conducted in the Department of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences (SKIMS) which is the largest tertiary care hospital in the Kashmir region. This study was conducted to see the prevalence of ADA testing in our laboratory. Retrospective data was collected from 2018 to 2021 of all ADA test assays done in the lab. Demographic characters like age, the sex were recorded. All types of body fluids received for ADA testing were analysed for the enzyme.

### 2. Methods

The body fluids received in our lab from 2020 till the present were tested using photometric enzymatic determination for ADA enzyme on the auto-analyzer (AU5800, Beckman Coulter Inc., CA, USA) by commercially available assay kit from Diasys (DiaSys Diagnostics., Maharashtra, India). Both ADA controls and calibrator (DiaSys Diagnostics., India) were run before each test to check its quality control. Reference range of enzyme among various body fluids used by the lab is given in Table 1.

### 3. Physician questionnaire

To analyse the frequency of testing, reliability, and impact on the beginning of treatment and continued management of suspected tuberculosis, a questionnaire was created in Google form and distributed to physicians from various disciplines (Table 2).

**Table1.** Reference range of adenosine deaminase assay levels for various fluids used in laboratory

| Sample type | Range (U/L) | Interpretation |
|---|---|---|
| Pleural fluid/Ascitic fluid/Serum/Pericardial fluid/Synovial fluid | <30 | Normal |
| | 30~40 | Suspected |
| | >40~60 | Strong suspected |
| | >60 | Positive |
| CSF | <9 | Normal |
| | >9 | Positive |

**Table 2.** Lists of questionnaire

| Questions | Options |
|---|---|
| 1. Do you consider ADA testing in suspected TB patients | • Always<br>• Occasionally<br>• Never |
| 2. How does ADA help you in the workup of patients with suspected TB | • Definitive diagnosis<br>• Supportive<br>• No role<br>• On presentation |
| 3. How early in your TB workup did you order ADA testing | • Within a week<br>• More than a week |
| 4. Do you start ATT based on positive ADA results | • Yes<br>• No |
| 5. If ADA is positive would you still go for another diagnostic testing | • Always<br>• Never<br>• Depends on the availability of the specimen |
| 6. What are the advantages of ADA testing | • Easily available<br>• Low cost<br>• Rapid results<br>• All of the above |
| 7. Common fluid sent | • Pleural<br>• Ascitic<br>• Serum<br>• CSF<br>• Pericardial |
| 8. Speciality | • Medicine<br>• Neurosurgery<br>• Thoracic surgery<br>• Pediatrics<br>• Neurology<br>• Others |

### 4. Statistical analysis

Data was recorded in Microsoft Excel and coded before analysis. Categorical data were presented as numbers and percentages while quantitative was expressed as mean±standard deviation. The distribution of ADA values in patients with respect to their age and gender was calculated using STATA-16 for Windows (StataCorp, College Station, TX, USA) in all types of fluids. Pearson's Chi-square test and Fisher exact test were used to calculate the two-way measure of association.

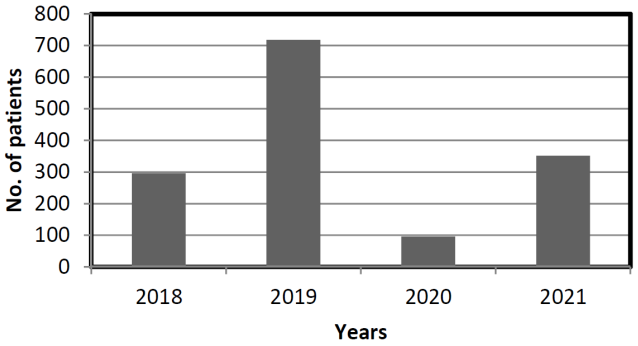


**Figure 1.** Numbers of patient samples was distribution by years.

## RESULTS

### 1. Characteristics of participants

A total of 1461 samples for ADA assay testing were received over a period of two years (Figure 1). The mean age of the study population was 56.69±11.7 years with a majority of them being males (55.72%).

### 2. Distribution of various body fluid received

The distribution of various body fluids received for ADA assay is shown in Tables 3-5. Pleural fluid constituted the maximum samples for ADA assay (N=817, 55.92%) followed by samples of ascitic fluid (N=231, 15.81%), cerebrospinal fluid (N=207, 14.16%), serum (N=141, 9.65%), pericardial fluid (N=26, 1.77%) and synovial fluid (N=12, 0.82%). Other fluids received include drain fluid, knee fluid, and pus (N=27, 1.84%).

### 3. ADA values with respect to age and gender

A total of 817 samples of pleural fluid were received out of which 114 (13.95%) were positive. The maximum representation of positive cases was from males within age group greater than 50 years (Tables 3, 4).

Among 231 ascitic fluid samples, only 20 samples (8.66%) were having ADA concentrations greater than 60 U/L (equal in both genders) with a maximum in age group greater than 50 years (Tables 3, 4).

A very less number of pericardial and synovial fluid samples was received by the laboratory (Tables 3, 4). Moreover, no patient was falling under the range of the suspect category. Only 2 male patients (age>50 years) showed ADA greater than cut-off value 60 U/L in the pericardial fluid sample.

Serum samples (N=141) for ADA analysis were being referred to the department. Frequency percentage distribution showed 13 (9.22%) patients were having ADA value greater than 60 U/L (169±125.60) more from age above 50 years (Table 4).

CSF samples (N=207) were tested for ADA enzyme activity and it was observed from the data that 56 (27.05%) patients were having enzyme values greater than cut-off (9 U/L) but again the maximum falls in the age group of above 50 years (Table 5).

## 4. Physicians response

Maximum responses were received from the specialty of General medicine. 76.9% of physicians order ADA testing within a week of admission. 84.6% of physicians consider ADA testing in suspected tuberculosis patients. 100% of physicians reported the supportive role of ADA levels in the workup of patients with suspected tuberculosis. 61.5% of responses support anti-tubercular treatment (ATT) therapy based on positive ADA results. If ADA comes positive, 84.6% of physicians recommend going for other diagnostic testing. In conclusion, 85% of

**Table 3.** Distribution of ADA values in patients in different fluids with respect to their gender N (%)

| Sample | Gender | Normal | Suspect | Strong suspect | Positive | *P*-value |
|---|---|---|---|---|---|---|
| Pleural fluid | Male | 340 (69.39) | 42 (8.57) | 35 (7.14) | 73 (14.90) | 0.1 |
| | Female | 234 (71.56) | 18 (5.50) | 34 (10.40) | 41 (12.54) | |
| Ascitic fluid | Male | 75 (74.26) | 3 (2.97) | 13 (12.87) | 10 (9.90) | 0.1 |
| | Female | 107 (82.31) | 6 (4.62) | 7 (5.38) | 10 (7.69) | |
| Serum | Male | 59 (88.82) | 3 (4.11) | 4 (5.48) | 7 (9.59) | 0.4 |
| | Female | 60 (88.24) | 1 (1.47) | 1 (1.47) | 6 (8.82) | |
| Pericardial fluid | Male | 11 (78.57) | 0 (0) | 1 (7.14) | 2 (14.29) | 0.3 |
| | Female | 11 (91.67) | 0 (0) | 1 (8.33) | 0 (0) | |
| Synovial fluid | Male | 4 (80.00) | 0 (0) | 0 (0) | 0 (0) | 0.6 |
| | Female | 5 (71.43) | 1 (14.29) | 0 (0) | 2 (20) | |

**Table 4.** Distribution of ADA values in patients in different fluids with respect to their age N (%)

| Sample | Age | Normal | Suspect | Strong suspect | Positive | *P*-value |
|---|---|---|---|---|---|---|
| Pleural fluid | <50 | 134 (71.66) | 11 (5.88) | 16 (8.56) | 26 (13.90) | 0.8 |
| | ≥50 | 440 (69.84) | 49 (7.78) | 53 (8.41) | 88 (13.97) | |
| Ascitic fluid | <50 | 42 (77.78) | 3 (5.56) | 6 (11.11) | 3 (5.56) | 0.6 |
| | ≥50 | 140 (79.10) | 6 (3.39) | 14 (7.91) | 17 (9.60) | |
| Serum | <50 | 46 (80.78) | 2 (3.51) | 3 (5.26) | 6 (10.53) | 0.7 |
| | ≥50 | 73 (86.90) | 2 (2.38) | 2 (2.38) | 7 (8.33) | |
| Pericardial fluid | <50 | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0.8 |
| | ≥50 | 20 (83.33) | 0 (0) | 2 (8.33) | 2 (8.33) | |
| Synovial fluid | <50 | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0.6 |
| | ≥50 | 7 (70) | 1 (10) | 0 (0) | 2 (20) | |

**Table 5.** Distribution of ADA values in patients in CSF with respect to their gender and age

| Gender | Normal | Positive | *P*-value | Age | Normal | Positive | *P*-value |
|---|---|---|---|---|---|---|---|
| Male | 85 (75.89) | 27 (24.11) | 0.3 | <50 | 33 (76.74) | 10 (23.26) | 0.5 |
| Female | 66 (69.47) | 29 (30.53) | | ≥50 | 118 (71.95) | 46 (28.05) | |

physicians will recommend ADA testing as it is a rapid, easily available test with low cost.

## DISCUSSION

Tuberculosis is a common infectious disease caused by MTB [13]. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It continues to be a major cause of morbidity and mortality worldwide. The diagnosis is usually based on clinical presentation, radiological findings, and positive tuberculin test. However, Clinicoradiological features are variable and the latter tests may be falsely negative [14]. The culture methods are time-consuming. The MTB- PCR is another reliable and fast test for the detection of tuberculosis but being a sensitive test it is prone to contamination. Moreover, PCR had few disadvantages like it needs more resources and sophisticated equipment, price is higher, needs longer time for test results, and not every hospital can set PCR laboratory [12]. Under these circumstances, drug therapy is started empirically. Among Lab diagnostic tests, ADA assay which is easy to perform, cheap, less time-consuming is therefore prescribed for initial management of tuberculosis and lastly for patients with extrapulmonary tuberculosis [15]. Besides other clinicopathological parameters, ADA level determination plays an important role in the diagnosis so physicians routinely prescribe it for patients to rule out tuberculosis [16]. ADA deficiency also results in impaired thymocyte development and B-lymphocyte immunoglobulin production resulting in severe combined immunodeficiency [17, 18]. ADA is also increased in various other infections like viral hepatitis, malignant tumors, typhoid, mononucleosis, leprosy, brucellosis, HIV infections. These diseases are usually excluded based on clinical presentation.

Our laboratory receives samples from different medical and surgical specialties especially from pulmonology, neurology, neurosurgery, thoracic surgery among others. Most of the specimens for ADA testing received by our lab were pleural fluid. Almost all researchers have shown sensitivity and specificity of 90% to 100% for the value of ADA in the pleural fluid using different cut-off levels [19]. An elevated level of ADA is seldom found in non-tuberculous pleural effusions. An ADA level of less than 40 U/L virtually excludes tuberculosis in lymphocytic pleural effusions [20]. In a study that investigated extrapulmonary TB cases, there was a statistically meaningful distinction in the ADA level in tuberculosis and non-tuberculosis disease, and the ADA level in extrapulmonary effusions had a considerable value, accuracy, and sensitivity in differentiating tuberculosis cases from non-tuberculosis cases. Therefore, In TB endemic countries, patients with raised ADA levels could be considered to have tuberculosis where acid-fast bacilli positivity is low [21]. Serum ADA activity is not very sensitive for the diagnosis of tuberculosis [22].

As far as our data is concerned it is from the ethnic population of the Kashmir region of India. Ethnicity can impact plasma biomarkers [23, 24]. There was no significant correlation found between ADA values with respect to their gender and age. But overall we observed that ADA value remained higher in patients greater than 50 years of age. And the male gender was in maximum number in most of the fluids received. Gender also impacts the immune system [25] and the male gender is associated with increased susceptibility to a range of infections [26, 27] and there is an interaction between sex and age [28].

In order to fully utilize this important biomarker, there is a need to improve its specificity and to minimize false positives and choose suitable cut-off values hence further research is needed.

In countries like India where there is a high burden of tuberculosis and limited possibility of using other methods like culture or PCR, ADA test can help to rule out TB. Physicians in these settings highly rely on ADA testing in combination with clinical and other laboratory finding to start early treatment.

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