

## Study of Serum Adenosine Deaminase (ADA) Level in Diagnosis of Extrapulmonary and Smear Negative Tuberculosis



### Biochemistry

**KEYWORDS :** Extrapulmonary TB, Pulmonary smear negative TB, Adenosine deaminase.

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### ABSTRACT

**Introduction:** Tuberculosis is a major cause of morbidity and mortality throughout the world. One-third of the world's population is infected with the TB, 5.2 million incident pulmonary TB patients notified globally in 2014. Only 3.0 million (58%) smear positive were bacteriologically confirmed, 42% of patients who were not bacteriologically confirmed were diagnosed clinically i.e. based on symptoms, chest X-Ray abnormalities or suggestive histologically and remains diagnostic challenge. In view of role of serum ADA in TB diagnosis this study was planned to investigate the diagnostic value of serum adenosine deaminase in diagnosis of extra-pulmonary negative tuberculosis (EPTB) and pulmonary smear negative tuberculosis (SNPTB), because rapid and accurate diagnosis is an important element of TB treatment and control.

**Objective:** To evaluate the diagnostic value and compare serum ADA activity in extra-pulmonary and pulmonary smear negative tuberculosis and to compare it with control.

**Method:** Total of 120 volunteers was enrolled in the study after obtaining informed written consent. They were divided into 3 groups. Group I includes 40 healthy Individuals; Group II 40 patients newly diagnosed with extra-pulmonary TB and Group III 40 patients newly diagnosed with pulmonary smear negative TB. Serum ADA was estimation by modified GIUSTI method.

**Result:** Serum ADA levels were significantly increased ( $p < 0.001$ ) in extrapulmonary TB ( $27.81 \pm 7.034$  U/L) and Pulmonary Smear Negative TB ( $35.12 \pm 12.1$  U/L) as compared to control ( $14.603 \pm 4.69$  U/L) with cut-off value 20 U/L.

**Conclusion:** Moreover our study being cost effective, highly reliable, reproducible, simple to perform and less time consuming; in addition it helps in rapid and accurate diagnosis of EPTB and pulmonary smear negative tuberculosis, it can be included in routine diagnosis and prognosis of tuberculosis.

### INTRODUCTION:

Tuberculosis (TB) is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years.<sup>[1]</sup> It is a major cause of morbidity and mortality throughout the world. One-third of the world's population is infected with the TB bacillus.<sup>[2, 3]</sup> The tuberculosis epidemic results in nearly two million deaths and nine million new cases per year, 95% in developing countries. In India 2.3 million cases are estimated to have occurred, accounting for approximately one fifth of the global incidence. The World Health Organization (WHO) 1990 report on the Global Burden of diseases ranked tuberculosis as the seventh most morbidity causing disease in the world, and expected it to continue in the same position up to 2020.<sup>[4]</sup>

Once infected active disease develops in about 10% of cases usually within 1-2 years after exposure from TB.<sup>[5]</sup> Some of the healthy subjects get contracts tuberculosis every four seconds and one of them dies every 10 seconds.<sup>[6]</sup> In 2006, about 1.4 million cases of tuberculosis were registered for treatment in India; 28.7% of them were new smear negative cases.<sup>[8]</sup>

The initial diagnostic approach to suspected cases of pulmonary tuberculosis is to demonstrate *Mycobacterium tuberculosis* in stained smears of expectorated sputum. In most of the tuberculosis centers, even after careful search, the bacteriological positive yield from sputum is around 16 to 50% and large portion remain negative in spite of clinical profile and radiological lesions being consistent with diagnosis of pulmonary tuberculosis.<sup>[9]</sup> However, 40 to 60% of patients with pulmonary disease and about 75% of patients with extra-pulmonary disease are smear negative, and in this situation even contemporary culture meth-

ods take several weeks to become positive.

There are different diagnostic methods but they have some limitations.<sup>[12,13,14,15]</sup> Pulmonary TB is usually diagnosed from clinical and radiological findings. The laboratory diagnosis is based on Ziehl-Neelsen (ZN) staining for acid-fast bacilli (AFB) and the growth of the causative organism *Mycobacterium tuberculosis* on Löwenstein-Jensen (LJ) culture,<sup>[16]</sup> which is the golden standard for TB diagnosis. However *Mycobacterium tuberculosis* grows very slowly, it can take up to six weeks to isolate it in culture. Determination of susceptibility to drugs can add another three to six weeks to process. Meanwhile the disease may progress and be transmitted to others when appropriate treatment is delayed and hospitalization increases.<sup>[17]</sup> ZN staining is rapid, easy to perform and inexpensive, but it lacks sensitivity which is already discussed. Problem arises when sputum smear result is repeatedly negative for acid fast bacilli.<sup>[18]</sup>

Polymerase Chain Reaction (PCR) is expensive and is not found to be more sensitive to pleural fluid. All these diagnostic tests including the newer Nucleic Acid Amplification, Interferon gamma and Lysozyme, either time-consuming, lack sensitivity or specificity or required technology is very intensive and expensive as well, thus limiting their usefulness and access especially in developing countries with insufficient resources.<sup>[19]</sup>

Adenosine deaminase activity (ADA) is also measured in diagnosis of TB and also in differential diagnosis of TB. Adenosine deaminase is an enzyme of purine catabolism; its activity has been found to be increased in various diseases such as tuber-

culosis, HIV, typhoid, infectious mononucleosis and certain malignancies especially those of hemopoietic origin. ADA assay in various body fluids had established its usefulness in the laboratory diagnosis of extrapulmonary TB (such as meningeal, pleural, peritoneal and pericardial TB) and SNPTB.<sup>[20]</sup>

By considering the importance of rapid and accurate diagnosis, in TB treatment and control, the present study was planned to investigate the diagnostic value of serum adenosine deaminase in diagnosis of EPTB and SNPTB and to compare it with control group.

**AIM:** To study serum adenosine deaminase (ADA) level in diagnosis of extrapulmonary and pulmonary smear negative tuberculosis.

**OBJECTIVES:** To evaluate the diagnostic value of serum ADA activity in extra-pulmonary and pulmonary smear negative tuberculosis and to compare serum ADA activity in study groups and control group.

**MATERIAL & METHODS:** The Institutional Ethics Committee approval was obtained before initiating the study. The study was conducted in the Departments of Biochemistry, Dept. of Respiratory Medicine, Dept. of Surgery, MGM Medical College & Hospital, Kamothe, Navi Mumbai. Present study is a prospective study completed over a period of 12 months i.e. from February 2014 to February 2015

**The volunteers are grouped into following three groups:-**

- Group I:** 40 healthy Individuals.
  - Group II:** 40 patients newly diagnosed with extra-pulmonary TB (EPTB).
  - Group III:** 40 patients newly diagnosed with pulmonary smear negative TB (SNPTB).
- Inclusion Criteria:** Following patients are included for the present study

Patients with Extra-pulmonary TB diagnosed by clinician.  
Extra pulmonary TB patient with smear positive histopathologically.

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.

**Exclusion criteria:** Following patients will be excluded from the study -

- No respiratory symptoms
- Normal chest X-rays.
- Liver diseases
- HIV/AIDS
- Gross congestive heart failure
- Typhoid fever
- Infectious mononucleosis
- Gout / Rheumatoid arthritis
- Skeletal muscle injury
- Renal failure
- Brucellosis. Bronchogenic carcinoma

**Study Procedure:** 2.0 ml of blood was collected by venepuncture. Serum was separated preserved at -60°C till the analysis. Serum ADA estimated by ADA kit (Kinetic Reaction), the modified **GIUSTI** method using semi auto analyzer.

**RESULTS:**

**Table 1: Gender wise distribution of healthy individuals, extrapulmonary TB and pulmonary smear negative TB**

| Groups              | Sex  |        | Total |
|---------------------|------|--------|-------|
|                     | Male | Female |       |
| Healthy Individuals | 32   | 8      | 40    |
| Extra Pulmonary TB  | 12   | 28     | 40    |

|                             |    |    |     |
|-----------------------------|----|----|-----|
| Pulmonary Smear Negative TB | 30 | 10 | 40  |
| Total                       | 74 | 46 | 120 |

**Tables 2: Comparison of subjects according to weight (Kg) in healthy individuals, extra pulmonary TB and pulmonary smear negative TB.**

| Groups                      | N  | Mean ± SD       |
|-----------------------------|----|-----------------|
| Healthy Individuals         | 40 | 62.97 ± 9.78    |
| Extra Pulmonary TB          | 40 | 47.1 ± 15.05**  |
| Pulmonary Smear Negative TB | 40 | 48.17 ± 10.01** |

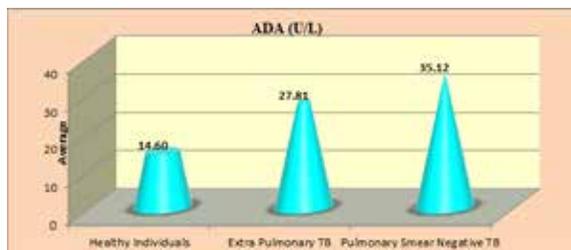
\*\*p<0.001 (highly statistically significant)

**Table 3: Comparison of ADA level (U/L) of healthy individuals, extra Pulmonary TB and pulmonary Smear negative TB.**

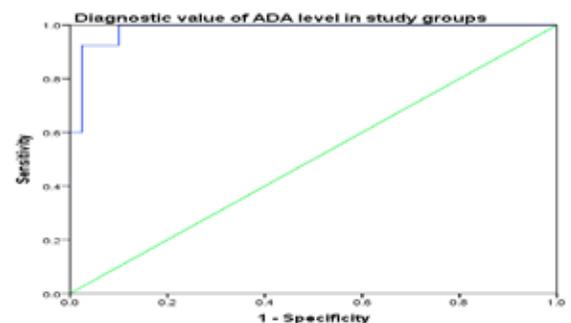
| Groups    | N                           | Mean ± SD          |
|-----------|-----------------------------|--------------------|
| ADA (U/L) | Healthy Individuals         | 40 14.603 ± 4.69   |
|           | Extra Pulmonary TB          | 40 27.81 ± 7.034** |
|           | Pulmonary Smear Negative TB | 40 35.12 ± 12.09** |

\*\*p<0.001 (highly statistically significant)

**Graph 1: Comparison of ADA levels (U/L) in different groups such as healthy individuals, extrapulmonary TB and pulmonary smear negative TB.**



**Graph 2: ROC (Receiver operating characteristic) curve for serum ADA in TB diagnosis**



**DISCUSSION:**

Tuberculosis (TB) is caused by Mycobacterium tuberculosis and is one of the most important infective causes of human mortality and morbidity worldwide.<sup>[21]</sup> India is the highest TB burden country with WHO statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB in India, out of a global incidence of 8.7 million cases. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB.<sup>[22]</sup>

The present study reveals that male subjects were more prone to pulmonary smear negative TB (4:1 male to female), whereas female were more prone to extrapulmonary TB (1:2.3 male to female) **Table 1.** Balasubramanian R *et al.*<sup>[23]</sup> reported same prevalence in their review i.e. Lymph node TB (LNTB) is the commonest form of EPTB. Peripheral LNTB have been in majority female, while pulmonary TB is more common in adult males.

The weight of control subjects was ( $62.97 \pm 9.78$  Kg), extrapulmonary subjects were ( $47.1 \pm 15.05$  Kg) and smear negative subjects was ( $48.17 \pm 10.01$  Kg). Our study shows that the study groups' patient weights is significantly declined as comparison of control group ( $p < 0.001$ ) but non-significant between study groups ( $p > 0.05$ ) **Table 2**.

Patients with Tuberculosis (TB) often suffer from severe weight loss, a symptom those are considered immuno-suppressive and a major factor of severity and disease outcome. Malnutrition is an important risk factor for TB, as cell-mediated immunity (CMI) is key host defense against TB and other factors such as socio-economic demographic characteristics, smoking and drinking habits.<sup>[23]</sup>

Serum ADA levels are compared in healthy subjects with extrapulmonary TB and pulmonary Smear negative TB. Serum ADA levels are significantly increased ( $p < 0.001$ ) in extrapulmonary TB ( $27.81 \pm 7.034$ U/L) and Pulmonary Smear Negative TB ( $35.12 \pm 12.1$  U/L) as compared to healthy individual. The results are concurrent with Agarawal MK *et al.*<sup>[24]</sup>, Rathod VS *et al.*<sup>[25]</sup>, Verma Met *al.*<sup>[26]</sup>, Stevanovic G. *et al.*<sup>[27]</sup> and Sharma D *et al.*<sup>[28]</sup>

Significantly increased serum ADA activity ( $p < 0.001$ ) in pulmonary smear negative TB and EPTB compared to healthy controls is due to activation of cell mediated immunity. In tuberculosis there are increased numbers of T-lymphocytes and macrophages, which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes. In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T-cells, may cause a high serum ADA activity in patients with TB.<sup>[29]</sup>

Serum ADA **cut-off value** is assessed from receiver operating characteristic (ROC) curve for extra pulmonary TB and pulmonary smear negative TB. The cut-off value 20 U/L and taken as

the best cut-off point. For this cut off value the sensitivity was 99 % and specificity 90% ( $p < 0.001$ ). The area under ROC Curve is 0.984 (95% CI-0.962-1.00). In present study none of the healthy individual showed ADA value above this limit and none of study subjects showed a lower value than 20 U/L. Our results are concurrent with Afrasiabian S *et al.*<sup>[30]</sup> and Stevanovic G. *et al.*<sup>[27]</sup>

ADA is the enzyme which is present in every cell. Highest concentration of ADA is seen in monocytes, macrophages and T-lymphocytes. These cells consist of ADA, 5-20 times more than B-cell and 10 times more than erythrocytes. Monocytes/macrophages are the main targets of mycobacterium tuberculosis, which multiply slowly inside the host cell. Then macrophages stimulate the cell-mediated immune response by releasing cytokines, which attract T-lymphocytes to the site. Defensive environment kill or limit the replication of pathogens and finally destroys the macrophages. Thus increased concentration of ADA may be found due to antigenic stimulation of phagocytic cell, its propagation, differentiation and macrophages destructions.

Elevated levels of ADA may depend on severity of TB, immune status and age. Limitation of present study is that increased serum ADA activity is also found in diseases of cell mediated immunity of unknown illness.

## CONCLUSION:

Significant increased concentrations of serum adenosine deaminase in study groups as compared to control group, aids to use ADA as a screening test. However, larger sample size is required to confirm reference values for serum ADA and to assess its diagnostic utility. The serum concentration of ADA may be used as a surrogate marker for EPTB and pulmonary smear negative TB and the findings should be correlated with clinical presentation of disease. Moreover our study being cost effective, highly reliable, reproducible, simple to perform and less time consuming; in addition it helps in rapid and accurate diagnosis of EPTB and pulmonary smear negative tuberculosis, it can be included in routine diagnosis and prognosis of tuberculosis.

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