

## **Evaluation of Serum adenosine deaminase activity during the course of pulmonary tuberculosis treatment**

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### **Abstract**

**Tuberculosis is an ancient human scourge that continues to be an important public health problem world wide. The risk of increasing spread of tuberculosis and development of drug resistance make early diagnosis a matter of utmost concern. The significance of serum ADA levels in the diagnosis of tuberculosis is known. However there is hardly any study literature that compares the serum ADA level during the treatment of the disease. Te objective of the study was to evaluate the effectiveness of Serum ADA in prognosis of pulmonary tuberculosis at different durations of the therapy in relation with other diagnostic tests of pulmonary tuberculosis. Serum ADA was measured along with other diagnostic markers during the course of treatment. The serum ADA values in the study group was (mean  $\pm$  SD)  $41.48 \pm 8.02$  U/L and in controls (mean  $\pm$  SD) of  $17.60 \pm 5.17$  U/L. After the 1<sup>st</sup> month of treatment there was no significant change ( $p > 0.05$ ) in serum ADA levels  $39.60 \pm 0.79$ U/L, however significant difference was recorded after the second month of treatment in serum ADA level  $29.66 \pm 0.83$ U/L and similarly at sixth month of treatment  $22.12 \pm 0.51$ U/L difference in serum ADA was significantly high. The difference in serum ADA levels between the first month and second month & first month and sixth month were statistically significant. ( $p < 0.001$ ,  $p < 0.001$  respectively). The measurement of serum ADA could be of significant help to evaluate the response to the therapy, particularly during second and sixth month. This may have an association with lymphocytic activation.**

**Key words:** Serum ADA, pulmonary Tuberculosis, prognostic markers

*Accepted November 17 2011*

### **Introduction**

India is the highest TB burden country accounting for one fifth of the global incidence (Global annual incidence estimate is 9.4 million cases out of which it is estimated that 1.98 million cases are from India) [1]. India is 17th among 22 High Burden Countries in terms of TB incidence rate. [2] In diagnosis of tuberculosis, microbiologic, genetic, immunologic and biochemical methods are used [3]. The measurement of adenosine deaminase (ADA) activity is one of the biochemical methods. ADA is an enzyme that converts adenosine to inosine, and deoxyadenosine to deoxyinosine in the pathway of purine catabolism, and by this way catalyses irreversible deamination [4]. ADA acts in proliferation and differentiation of lymphocyte and especially T lymphocyte [5]. However there is no study literature that compares the serum ADA level with tuberculosis categories or investigates the relation of the serum ADA levels with response to anti-tuberculosis therapy. Therefore, this study was aimed to

examine the level of serum ADA in patients undergoing anti tubercular treatment at different intervals.

### **Objectives:**

To know the effectiveness of serum ADA in the prognosis of pulmonary tuberculosis at different durations of therapy in relation with Known prognostic markers of pulmonary tuberculosis.

### **Material and Methods:**

The Prospective study was carried out in Navodaya medical college hospital & research centre, Raichur between April 2009 & July 2010.

### **Locus of the study:**

The study was conducted in the department of Biochemistry NMC, Raichur and Biochemistry section, Microbiology & Pathology sections of central clinical laboratory of

Navodaya Medical College Hospital and Research Centre, Raichur, Karnataka.

### **Study Group**

A total of 44 clinically and laboratory diagnosed pulmonary tuberculosis patients, who have consented to participate in the study during treatment were selected. The study and data collection were carried out with the approval from institutional ethical committee and informed consent were taken from all the study subjects. Of 44 cases 32 were males (72.72%) and 12 were female (27.27%) cases, the mean age group was  $42.96 \pm 15.29$ .

### **Control Group**

A total of fifteen normal healthy individuals (paramedical and medical staff of Navodaya medical college) aged between 20 years - 50 years [11 Males (73.3%) and 4 females (26.66%)] were selected as controls after getting their consent for participation.

**Inclusion and exclusion criteria followed in the present study are as follows:**

#### ***Inclusion criteria for pulmonary TB***

Cases diagnosed as a "new case" of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli; Radiographic abnormalities consistent with pulmonary tuberculosis.

Only those freshly diagnosed pulmonary tuberculosis cases, who were willing to participate during treatment were included in the study group.

#### ***Exclusion criteria for pulmonary TB***

Patients with extra pulmonary TB and /or patients requiring surgical intervention,

Chronic pulmonary TB (receiving at least two courses of anti-TB treatment for more than six months), and drop-outs were excluded from the study group.

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

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 and CRP estimation. ESR by Westergren's method [6], Quantitative determination of C-Reactive Protein (CRP) [7,8,9] by Turbidometric Immunoassay using ERBA Chem. 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 [10,11]. Adenosine deaminase activity was determined in serum by using ADA MTB diagnostic kit from Microexpress a division of Tulip diagnostics (P) Ltd.

### **Statistical Analysis**

The results were expressed as mean  $\pm$ SEM (Standard error of mean) taking 95% CI. Analysis of variance (ANOVA) followed by Bonferroni Multiple Comparison Test was used for statistical evaluation. P values less than 0.05 were considered as significance and  $p < 0.01$  as highly significant. Correlation matrix between normally distributed indices was determined using the Pearson correlation coefficient.

## **Results**

### ***Control Group***

In this group the serum ADA activity level ranged from 12.43 to 22.73 U/L with a mean value (mean  $\pm$  SD) of  $17.60 \pm 5.17$  U/L.

### ***Study Group***

The serum ADA values in study group before treatment ranged between 33.46 U/L and 49.50 U/L with the mean  $\pm$  SEM serum ADA activity of  $40.22 \pm 0.79$  U/L. After first month of treatment values are  $39.60 \pm 0.79$  U/L followed by second and sixth month with a mean of  $29.66 \pm 0.83$  U/L and  $22.12 \pm 0.51$  U/L respectively as shown in table-1.

After 1<sup>st</sup> month of treatment there was no significant ( $p > 0.05$ ) change in serum ADA  $39.60 \pm 0.79$  U/L levels. However highly significant difference of  $29.66 \pm 0.83$  ( $p < 0.001$ ) was recorded after the second month of treatment. Similarly at sixth month of treatment difference in serum ADA ( $22.12 \pm 0.51$ ) was significantly high ( $p < 0.001$ ). The difference in serum ADA levels between the first month and second month & first month and sixth month were statically significant. ( $p < 0.001$ ,  $p < 0.001$  respectively) as shown in Figure-1.

C-reactive protein values before treatment have shown decrease from  $74.14 \pm 3.86$  to  $48.40 \pm 2.99$  after first month,  $21.24 \pm 1.55$  after second month and  $6.32 \pm 0.59$  mg/l after sixth month. Similarly ESR values have shown significant decrease from  $73.16 \pm 1.97$  before treatment to  $54.50 \pm 1.97$  after first month,  $36.55 \pm 1.65$  after second month and  $14.38 \pm 0.64$  mm/hr after sixth month.

There was a significant decrease in erythrocyte sedimentation rates and C-reactive protein values after the 2<sup>nd</sup> and

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6<sup>th</sup> month of treatment corresponding with the serum ADA levels as shown in Figure 2.

Lymphocyte count showed 1331 ± 50.49 before treatment, 1625.5± 48.65 after first month, 1389.7± 44.7 after

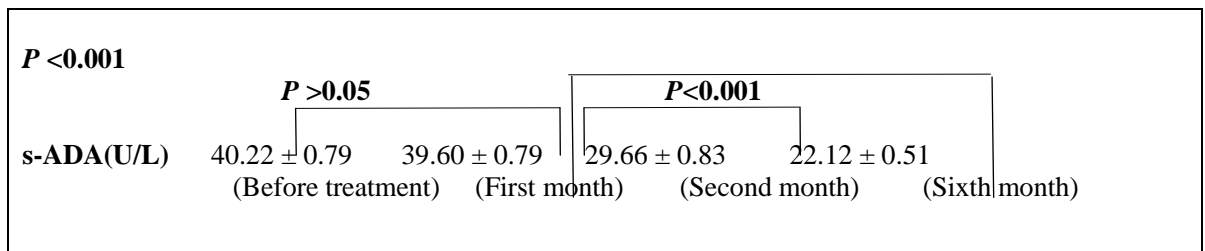
second month and 1719.7± 43.81 after sixth month of treatment. A positive correlation and trend between serum ADA and lymphocyte count was found after 1<sup>st</sup>, 2<sup>nd</sup> & 6<sup>th</sup> month of treatment as depicted in Figure -3,4&5.

**Table 1** Serum ADA and CRP levels, ESR & peripheral blood leukocyte and lymphocyte counts at before treatment and at one, two and six months after treatment (Mean ± SEM)

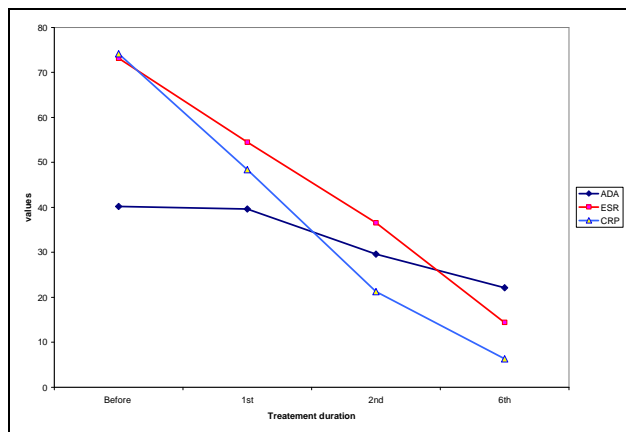
	B.T.	1 M	2 M	6 M	P-value
<b>s-ADA U/L</b>	40.22 ± 0.79	39.60 ± 0.79**	29.66 ± 0.83	22.12 ± 0.51**	P<0.0001
<b>ESR mm/hr</b>	73.16 ± 1.97**	54.50 ± 1.97**	36.55 ± 1.65	14.38 ± 0.64**	P<0.0001
<b>CRP Mg/L</b>	74.14 ± 3.86**	48.40 ± 2.99**	21.24 ± 1.55	6.32 ± 0.59**	P<0.0001
<b>Leukocyte count mm</b>	9322.2 ± 230.62	9116.6 ± 238.83*	8168.4 ± 245.77	7087.9 ± 239.29**	P<0.0001
<b>Lymphocyte count</b>	1331 ± 50.49**	1625.5 ± 48.65**	1389.7 ± 44.37	1719.7 ± 43.81	P<0.0001

\* p<0.05, \*\*p<0.01, Bonferroni Multiple Comparisons Test, If the value of t is greater than 2.419 then the P value is less than 0.05.

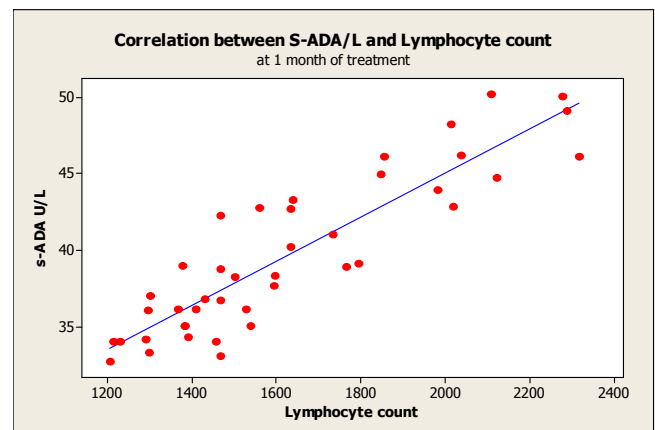
SEM: Standard error of mean



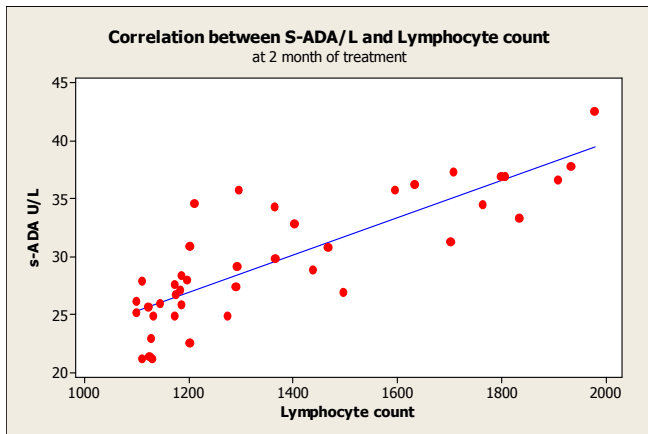
**Figure-1** The Course of serum ADA levels



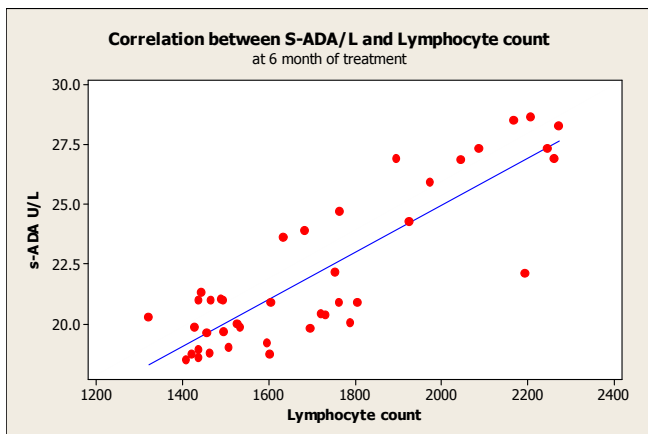
**Figure 2.** Trends of Serum ADA, ESR and CRP during the treatment of Pulmonary tuberculosis



**Figure 3.** Correlation between s-ADA U/L and Lymphocyte count after 1<sup>st</sup> month  
Pearson correlation of s-ADA U/L and Lymphocyte count = 0.895 P-Value = 0.000



**Figure 4.** Correlation between s-ADA U/L and Lymphocyte count after 2<sup>nd</sup> month  
Pearson correlation of s-ADA U/L and Lymphocyte count = 0.832 P-Value = 0.000



**Figure 5.** Correlation between s-ADA U/L and Lymphocyte count after 6<sup>th</sup> month  
Pearson correlation of s-ADA U/L and Lymphocyte count = 0.856 P-Value = 0.000

## Discussion

The values of serum adenosine deaminase were significantly higher in the study group than in the control group. After the first month of treatment of pulmonary tuberculosis, there was no significant change ( $p > 0.05$ ) in serum ADA when compared to initial reading, but after the second month & sixth month of treatment there was a significant decrease in the serum ADA values ( $p < 0.05$ ). However serum CRP and ESR values also decreased immediately after the first month of treatment (Figure-2).

In our previous work on serum ADA activity in pulmonary tuberculosis patients, we found that serum ADA was elevated in 88% of study group compared to control group followed by chest x-ray (76%) and sputum AFB (63%). And also significantly high serum ADA was noted

in pulmonary tuberculosis cases compared to non tuberculosis pulmonary diseases. [11]

The highest level of serum adenosine deaminase in patients with pulmonary tuberculosis corresponds to the severity of the disease, high bacterial isolation, the extent of destructive and infiltrative changes in lung tissue, and endotoxemia. This indicates that ADA measurement is an additional criterion for assessing the health status in pulmonary tuberculosis patients and the magnitude of destructive processes. Moreover, the measurement is of prognostic value as it provides an objective assessment of the course of an acute progressive process and the efficiency of chemotherapy used [12].

A study by Zafer Kartaloglu et al [13] showed a slight elevation of serum ADA in the first month, but it decreased during treatment in parallel with the effectiveness. A study by Meftun Unsal et al [14] revealed gradual decrease in serum ADA from  $30.1 \pm 11.7$  U/L to  $24.8 \pm 15.6$  after first month of treatment & after second month  $22.0 \pm 10.6$  in limited lesion cases. And in extended lesion cases before treatment ADA values was  $31.3 \pm 18.3$ , first and second month values were  $27.5 \pm 13.0$  and  $27.1 \pm 12.2$  U/L respectively.

Suzuki K et al [15] reported that the serum acute phase reactants decreased to normal levels within three to five weeks after negative results for tubercle bacilli were obtained in the sputum cultures. In addition, hematological abnormalities were observed in patients, such as normocytic normochromic anemia, leukopenia, neutropenia, and lymphocytopenia. These abnormalities reverted to normal with antituberculosis treatment [16]. Studies on lymphocyte subgroups reported that especially CD4<sup>+</sup> T cells showed an increase after the beginning of treatment [17, 18]. Callazos et al [19] was observed that a decrease in both the leukocyte and the lymphocyte counts paralleled the decrease in s-ADA levels after the first month of treatment. In our study the lymphocyte count and s-ADA levels showed a parallel course for two months. After the second month the lymphocyte count increased, while s-ADA levels continued to decrease. The levels of s-ADA showed no significant change ( $p > 0.05$ ) in the first month of the treatment and decreased after the second month. Perhaps this pattern is related to lymphocytic activation and/or a complex cytokine network regulating the lymphocytes. It is known that the course of the ADA level is associated not only with lymphocytes, but also some chemical mediators secreted by these cells [21]. The disease is under control after the second month, and the lymphocytes and cytokines normalize. Although lymphocyte numbers increased at the sixth month, s-ADA levels decreased. It could be thought that the patients who had lymphopenia in the beginning showed increase in lymphocyte count after the treatment and influences the change in s-ADA level. The lymphocyte counts and serum ADA levels demonstrated a parallel course in the

first two months and then they had different course. CRP & ESR reduced quickly after first month of treatment. The levels of serum ADA showed decrease after first month of treatment. This decrease in s-ADA after the first month might reflect the normalization of the altered lymphocyte turnover induced by tuberculosis. Therefore measurement of serum ADA could be of significant help to evaluate the response to therapy, particularly in those patients with increased value of the enzyme.

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