

# Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis

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**Background:** In some studies, the level of adenosine deaminase (ADA) in sputum and effusion liquids was used for the diagnosis of tuberculosis (TB). But it is not always possible to access these materials. The goal of this study is to assess the diagnostic value of serum ADA levels in pulmonary TB patients. **Materials and Methods:** In this study, 40 sputum smear-positive TB patients who were hospitalized and 40 non-TB patients who referred for surgeries were selected. A serum sample was collected and serum ADA level was measured by ADA kit. **Results:** The average (SD) of serum ADA in TB and non-TB patients were 20.88 ( $\pm 5.97$ ) and 10.69 ( $\pm 2.98$ ) U/L, respectively ( $P$  value  $< 0.05$ ). The best cut-off point was 14 U/L. The calculated area under the receiver operating characteristic (ROC) curve was 0.955 (95% CI, 0.914-0.995); sensitivity was 92.7% (95% CI, 84.7-100) and specificity was 88.1% (95% CI, 78.3-97.8) ( $P < 0.001$ ). **Conclusion:** Serum ADA level may be proposed as a proper index for TB diagnosis.

**Key words:** Adenosine deaminase, diagnosis, tuberculosis

## INTRODUCTION

Tuberculosis (TB) is one of the main reasons of mortality and morbidity globally and it kills about two million people annually.<sup>[1,2]</sup> Recent studies showed extensive delay in TB diagnosis.<sup>[3,4]</sup> There are different diagnostic methods but they have some drawbacks. To prepare mycobacterium culture, which is the golden standard for TB diagnosis, it may take 8 weeks. Finding acid-fast bacilli is the quick screening method for pulmonary TB diagnosis; nevertheless, its sensitivity is low. The polymerase chain reaction (PCR) test for TB diagnosis is expensive and it requires skilled personnel and lot of equipments. Therefore, in recent years, there has been a great demand for finding new microbiological, genetic, immunological, and biomedical diagnostic methods to diagnosis TB quickly and accurately. Measuring of adenosine deaminase (ADA) activity is a biomedical method.<sup>[5,6]</sup> ADA is an enzyme which contributes in purin metabolism.<sup>[7]</sup> ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity<sup>[5]</sup> and previous studies have proved its value in TB diagnosis, even for assessing TB effusions.<sup>[8,9]</sup> Activity of this enzyme increases in TB patients.<sup>[9]</sup> In some studies, the level of ADA in sputum and serum was used for diagnosis of TB, and it was monitored during TB treatment.<sup>[10-12]</sup> However, previous studies used effusion fluids and a very limited number of studies used patients' serum.<sup>[5-10,12]</sup> It is not

always possible to access effusion liquids everywhere in pulmonary and extra-pulmonary TB; therefore, it would be helpful to take advantage of serum levels. The goal of this study is to assess the diagnostic value and cut-off point of serum ADA levels in pulmonary TB patients.

## MATERIALS AND METHODS

It was a cross-sectional study (2011), which is conducted in Tohid Hospital (University Referral Hospital) in Sanandaj in Iran. In our study, case group included 40 sputum smear-positive TB patients who were admitted in infectious disease ward of the hospital. Inclusion criteria for TB patients were: "Having 2 or 3 sputum positive smears," or "one sputum positive smear and one positive sputum culture," or "one positive TB microbe smear sputum and suspected chest radiography."<sup>[13]</sup>

In addition, 40 non-TB patients, referred to Tohid Hospital in Sannandaj for surgeries, were selected as the control group. Inclusion criteria for control group were: Not having any TB patient in their family, not having history of close contact with TB patients, not having any infectious disease (according to the interview and their records), not having fever or any symptom of illness or of being toxic, normal cell blood count, and normal chest radiography.

After diagnosis of TB (before initiation of treatment), blood samples and patients' approval of consent form (research project number: 1387/87) were taken

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from TB group (at 7 AM). Then samples were centrifuged and serum ADA levels were measured by ADA kit manufactured by Diazyme Laboratories Company. In the first step, adenosine was affected by ADA and it becomes de-ammonized and shifted to inosine and then ammoniac was released. In the second reaction, because of glometat, released  $\text{NH}_3$  had become dehydrogenized and when it got close to allosteric activators, it combined with Nicotinamide adenine dinucleotide phosphate Hydrogen (NADPH) and released Nicotinamide adenine dinucleotide phosphate (NADP). Consequently, there was a direct relationship between activity (density) of ADA enzyme and speed reduction of radiation absorption in 340 nanometer wavelength (as NADPH changed to NADP+); it was measured by Diazyme Adenosine Deaminase Assay Kitt (Diazyme Laboratory; USA). Then, data were analyzed using Statistical Package for the Social Sciences (SPSS) 11.5 (Chicago, USA) software and ROC curve was plotted. In addition, sensitivity, specificity, and confidence interval of 95% were calculated.

## RESULTS

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. The most common age group for TB patients was 50s and for control group, it was 40s. Age average was 59 ( $\pm 13.5$ ) in TB patients and it was 49 ( $\pm 15.6$ ) for non-TB patients. The average of serum ADA in TB patients group and control group was 20.88 ( $\pm 5.97$ ) and 10.69 ( $\pm 2.98$ ), respectively. The best cut-off point was 14 U/L in which sensitivity and specificity were 92.7% (95% CI, 84.7-100) and 88.1% (95% CI, 78.3-97.8), respectively. The positive and negative predicative values were 88.4% (95% CI, 75-95.1) and 92.5% (95% CI, 79.6-98.4), respectively [Figure 1]. The calculated area under the ROC curve was 0.955 (95% CI, 0.914-0.995) ( $P < 0.001$ ).

## DISCUSSION

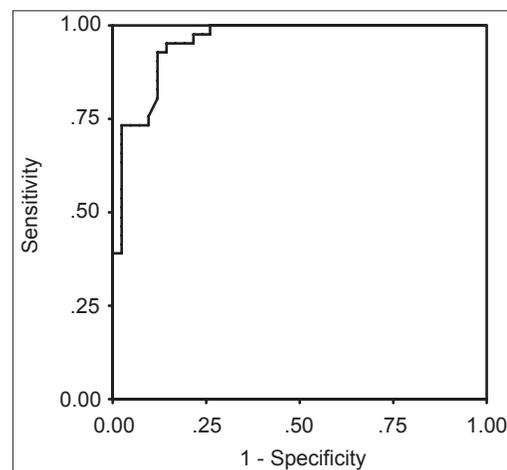
In this study, serum ADA level was an appropriate index for diagnosing smear-positive TB. High sensitivity and specificity were observed in serum level of 14 U/L. Therefore, serum ADA could be also used for diagnosis of pulmonary TB. Moreover, in this study, all TB patients had a serum level of more than 22.5 U/L. Hence, for suspicious cases of TB, increased levels of ADA could facilitate diagnosis.

Diagnostic value of serum ADA in pulmonary TB has been assessed only in few numbers of studies. Pairs *et al.*<sup>[7]</sup> reported an increase in ADA level in TB pleural effusion; other studies have also confirmed such an increase in TB pericardial effusions, peritoneum, and central nervous

system (CNS).<sup>[11,14,15]</sup> The main reason for the increased ADA levels in pleural effusion is the movement of T lymphocytes toward this area. Increase in ADA level is the result of a tropical inflammatory reaction caused by monocytes and macrophages.<sup>[11,16]</sup> When alveolar macrophages are infected by mycobacterium, this enzyme could be found in serum during active pulmonary disease. When TB infection is controlled, growth-markers of lymphocytes decrease; leucocytes will decrease in serum ADA levels concurrent with the decrease in lymphocytes. Because of this, serum ADA level could be utilized as a treatment response index.<sup>[10-12]</sup>

In Agarwal *et al.*'s study,<sup>[17]</sup> ADA level was 15.3 ( $\pm 0.23$ ) in healthy people, 19 ( $\pm 0.68$ ) in non-pulmonary TB cases, and 38.48 ( $\pm 1.56$ ) in pulmonary TB patients. In Jhamaria *et al.*'s study,<sup>[6]</sup> the average of serum ADA level was 19.9 U/L ( $\pm 2.99$ ) in control group, 43.95 U/L ( $\pm 2.48$ ) in sputum smear-positive people with typical or progressive disease, and 42.09 U/L ( $\pm 1.46$ ) and 40.02 U/L ( $\pm 2.58$ ) in negative sputum patients with mild or typical disease. In their study, in the cut-off point of 33 U/L, sensitivity and specificity were 98% and 100%, respectively. It seems that as the disease progresses, ADA levels increase; this subject was not considered in our study. In another study with an ADA level of 26.2 U/L, sensitivity, specificity, and positive predictive value were 95%, 83.3%, and 79.2%, respectively.<sup>[18]</sup>

In Gupta *et al.*'s study,<sup>[19]</sup> sensitivity, specificity, positive predictive value, and negative predictive value were 92.8%, 90%, 92.8%, and 90%, respectively, for diagnosis of TB in pleural effusion with an ADA level of more than 40. In Conde *et al.*'s study,<sup>[20]</sup> ADA level of 14 U/L was chosen as cut-off point. Stevanovic *et al.*<sup>[12]</sup> assessed serums of extra-pulmonary TB patients and in a cut-off point of 24, sensitivity and specificity were 94.3% and 92.2%, respectively; in their study, serum ADA level decreased as treatment started. In Dilmac *et al.*'s study,<sup>[21]</sup> serum ADA



**Figure 1:** Receiver operating characteristic curve for serum adenosine deaminase in tuberculosis diagnosis. Area under curve was 0.955

level in pulmonary TB patients was reported as 27.5 ( $\pm 11$ ) and it was 23.9 ( $\pm 24$ ) in Chronic Obstructive Pulmonary Disease (COPD) patients. In Rasolinejad's study,<sup>[22]</sup> serum ADA level was 21.51 in pulmonary TB patients and 11.47 in healthy people; in cut-off point of 14.5 U/L, sensitivity and specificity were 82% and 80.6%, respectively. In Lakshmi *et al.*'s study,<sup>[23]</sup> average ADA level was 13.3 U/L in negative smear and negative tuberculin patients, 33.52 ( $\pm 15.22$ ) in smear-positive and purified protein derivative (PPD) positive patients, and 16.5 ( $\pm 3.18$ ) in volunteer healthy people. Such differences may be due to TB severity, age groups, genetic differences, and dissimilarities in control groups. Therefore, further studies for identifying normal ADA levels in different societies may be useful.

Fortunately, in some autoimmune patients like rheumatoid arthritis, synovial ADA level is normal and it is similar to control group.<sup>[24]</sup> Thus, in autoimmune diseases that involve lung, ADA level could be used for TB differentiation. In some studies, ADA2 was also considered a useful tool for diagnosis;<sup>[16]</sup> it needs further studies.

## CONCLUSIONS

According to this study, serum ADA level is proposed as a proper index for TB diagnosis; in a cut-off point of 14, its sensitivity and specificity are calculated as 92.7% and 88.1%, respectively.

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