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The Relationship Between Serum Adenosine Deaminase Levels in Lung Tuberculosis Along with Drug Resistance and the Category of Tuberculosis

Filiz Çimen[1], Tansu Ulukavak Çiftçi[2], Bahadır M. Berktaş[1], Tuğrul Şipit[1], Nevin Taci Hoca[1], Güngör Dulkar[1]

[1]Atatürk Chest Disease and Chest Surgery Training and Research Hospital, Ankara, Turkey
[2]Gazi University, Faculty of Medicine, Department of Respiratory Medicine Ankara, Turkey

Abstract

Setting: The significance of adenosine deaminase (ADA) level in diagnosis of tuberculosis is known.

Objectives: We aimed to investigate serum ADA levels in patients with tuberculosis and its relation with drug resistance and tuberculosis categories.

Design: The study involved 51 pulmonary tuberculosis patients and eleven healthy controls. All patients classified according to the World Health Organization (WHO) and 60.8% of the patients were category I, 21.6% were category II and 17.6% were category IV pulmonary tuberculosis. Serum ADA levels of the sensitive cases to isoniazid (H), rifampicin (R), ethambutol (E) and streptomycin (S) were compared with those of the resistant cases.

Results: Serum ADA levels were higher in R and E sensitive group then in the resistant group (p=0.046, p=0.045). Serum ADA levels were similar in H and S sensitive group and resistant group (p>0.05). Serum ADA levels were higher in category I group when compared with the levels of the healthy group (p: 0.03). Comparison between the serum ADA levels of the groups of category I, II and IV with each other showed that the values of category I were significantly higher than values of category II (p=0,038). Although there were no statistically significant differences, it was shown that when the number of resistant drugs increased, the mean serum ADA level tend to decrease.

In conclusion, serum ADA levels of category I pati ents were higher than healthy group and while the number of resistant drugs increasing, ADA levels were decreasing

Key words: Pulmonary tuberculosis, adenosine deaminase, drug resistance

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INTRODUCTION

Epidemiological studies have shown that tuberculosis is a disease that endangers health of community with increasing incidence rate. In diagnosis of tuberculosis, microbiologic, genetic, immunologic and biochemical methods are used [1]. The measurement of adenosine deaminase (ADA) activity is one of the biochemical methods.

Corresponding Author: Filiz Çimen, Atatürk Chest Disease Education and Research Disease, Chest Disease, Ankara, Turkey Phone: +90 312 355 21 10 E-mail: fhcimen@yahoo.com

ADA is an enzyme that converts adenosine to inozine, and deoxiadenozine to deoxiinozine in the pathway of purine catabolism, and by this way catalyses irreversible deamination [2]. ADA acts in proliferation and differentiation of lymphocyte and especially T lymphocyte [3]. It acts in maturation of monocytes and transforming them to macrophage. It is a significant indicator of active cellular immunity [2-9]. The level of serum ADA increases in various diseases in which cell immunization is stimulated [8].

The significance of ADA level in diagnosis of tuberculosis is known. Previous studies have confirmed the diagnostic value of ADA activity in effusions due to pleural, pericardial, meningeal, and peritoneal tuberculosis especially in countries with high tuberculosis prevalence [6]. Drug resistance is seen frequently in category 2 and category 4 patients. It is thought that defect in cellular immunity followed by decreased ADA levels is seen with drug resistance. WHO distinguished tuberculosis patients in four diagnostic categories. Category I patients were defined as new smear (+) cases and / or severe forms of extra pulmonary tuberculosis. Category II patients were defined as previously treated sputum smear-positive cases that return with relapse, treatment after interruption or treatment failure. Category III patients were defined as new smear negative pulmonary tuberculosis and / or less severe forms of extra pulmonary tuberculosis. Category IV patients were defined as chronic and Multi Drug Resistant tuberculosis patients [10].

However there is no study in literature that compares the serum ADA level with tuberculosis categories or investigates the relation of the serum ADA levels with anti-tuberculosis drug resistance status. Therefore, this study aimed to examine the level of serum ADA in patients with various tuberculosis categories and to investigate its relation with drug resistance.

MATERIAL AND METHOD

This study was undertaken between June 1999 and December 2001 on 51 male patients with pulmonary tuberculosis who had completed their bed-treatment term in Ata-

türk Chest Disease and Chest Surgery Training and Research Hospital. The cases were selected from hospitalised patients admitted to our clinic consecutively. The eleven healthy male as control group was selected from volunteers among the hospital staff. Postero-anterior chest radiograph, full blood count, sedimentation, biochemistry, serum ADA, acido-resistance bacilli (ARB) smear and culture of sputum tests of the patients were studied.

The specimens of sputum were shown in tuberculosis culture, and after proliferation isoniazid (H), rifampicin (R), ethambutol (E) and streptomycin (S) resistance tests were carried out. Antibiogram was performed on the sputum of remaining 51 patients. The antibiograms were evaluated by indirect proportion method. These patients were classified according to the definitions of World Health Organization (WHO), and of them 60,8% (n=31) were accepted in category I, 21,6% (n=11) category II, and 17,6% (n=9) category IV.

In order to determine the pre-treatment serum ADA levels of patients, 5cc venous blood samples were obtained and centrifuged for 10 minutes in 3500 rpm. ADA activity was measured by Giusti method [11]. The reference value for normal serum ADA level is 14-20 U/l.

The people with liver disease, hematological malignity, and other lung diseases like sarcoidosis, pneumonia, empyema, Familial Mediterranean Fever, brucellosis, infectious mononucleosis, and rheumatoid arthritis were not included in the study.

Statistical analyses were performed using Mann Whitney U test, Exact test and Kruskal Wallis test. Two-tailed p value < 0.05 was considered statistically significant. Analyses of the results were performed by SPSS (SPSS Inc, Chicago IL) software.

RESULTS

The cases classified according to WHO categories, and the resistance status of the patients to drugs and ADA levels were compared in antibiogram group.

The mean ages of patients according to categories were as follows; 43.1 years in Category I , 46.6 years in Category II, and 43.8 years in Category IV. There was no statistically significant differance between the groups according to mean ages (p=0.723). Mean age of control group was 36.3. While there were no difference about sex in control and treatment group, there were statistically important difference about age in two groups (p=0,310).

Antibiogram was performed on the sputum of 51 patients. According to the antibiogram result 13 patients were resistant to S, 12 to R, 10 to H and 4 to E (25.5%, 23.5%, 19.6%, 7.8% respectively). Among all the patients, 58,8% (n: 30) were susceptible to all drugs, while 21,6% (n: 11) was resistant to a single drug; 5.9% (n: 3) to two drugs; 11,8% (n: 6) to three drugs, and 2% (n: 1) to four drugs.

When the serum ADA levels of individually H, R, E and S susceptible groups and resistant groups were compared, serum ADA level in R and E susceptible group was significantly higher than that of the resistant cases. (p=0.046, p=0.045) (Table 1).

Table 1. The comparison of serum ADA levels of the sensitive and resistant groups to each of the drugs.

	Serum ADA Level (U/l)				
Drug	**Susceptible (n:30)**		**Resistant (n: 21)**		
	Median	**Interquartile Range**	**Median**	**Interquartile Range**	**p**
H (isoniazid)	33.80	26.90	28.76	20.08	0.380
R (rifampicin)	33.80	17.14	23.07	10.71	0.046
E (ethambutol)	29.70	16.43	16.35	4.76	0.045
S (streptomycin)	35.05	17.57	24.18	9.94	0.133
Any*	36.60	29.13	26.00	16.80	0.235

* Susceptible to all drugs vs resistant to at least one drug.

Serum ADA levels of category I patients were found significantly higher than those of the healthy group (p=0.036), (Table 2, Figure 1). Comparison between the serum ADA levels of the groups of category I, II and IV with each other showed that the values of category I were significantly higher than values of category II (p= 0,038).

The serum ADA level means according to the number of drugs the patients were resistant to and dual comparisons within them are shown in Table 3.

There were no statistically significant correlation between mean serum ADA levels and the number of drugs to which the patients were resistant (p>0.05). The ADA levels

Table 2. Serum ADA levels according to the TB categories

	Serum ADA level (U/l)	
Category	**Mean**	**Standard Deviation**
Healthy Group	16.60	6.08
I(n=31)	29.03	18.73
II(n=11)	18.80	13.29
IV(n=9)	25.33	10.88

Serum ADA levels of category I patients were found significantly higher than those of the healthy group (p<0.05)

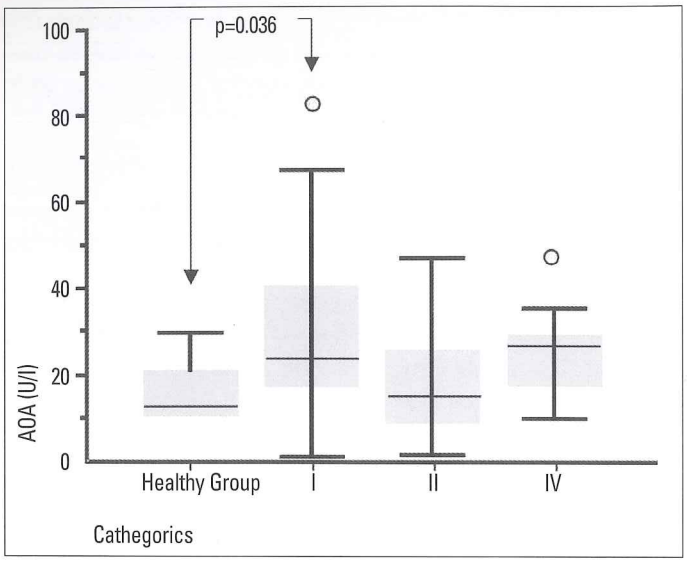

Figure 1. The comparison of serum ADA level means between diagnostic categories

Table 3. Mean Serum ADA levels according to resistant drugs

Number of resistant drug	Mean Serum ADA level ± SS
Cases resistant to 4 drugs(n=1)	17.5±0
Cases resistant to 3 drugs(n=6)	18.8±6.92
Cases resistant to 2 drugs(n=3)	24.8±7.81
Cases resistant to 1 drugs(n=11)	35.1±19.03
Cases susceptible to all drugs(n=30)	32.93±16.54

of the patients resistant to 3 and 4 drugs were significantly lower than the ADA levels of the patients with no resistance to drugs. Although there were no statistically significant differences, it was shown that when the number of resistant drugs increased, the mean serum ADA level tend to decrease.

DISCUSSION

The activity of ADA increases in pleural, pericardial, peritoneal and central nervous system tuberculosis [12-16]. Piras et al were the first to report increased ADA level in pleural effusion in tuberculosis pleuritis. Later studies have shown that a high level ADA could be determined in pleural effusion in rheumatoid arthritis, malignancy , parapneumonic effusions and empyema [8]. It has been reported that serum ADA level increases in liver diseases, tuberculosis, thymus diseases, infectious mononucleosis, malignancy (particularly hematological malignancy), sarcoidosis, acute leukemia, brucellosis, mediterranean spotted fever, pneumonia and rheumatoid arthritis [8,9, 17-20].

Immunologic reactions developing during tuberculosis infection are rather complex. The increase of ADA in pleural effusion is explained with the attack of T-lymphocyte to this region. Increase in ADA activity is the result of a local inflammatory response and it is mainly produced by monocytes and macrophages [21].

The serum ADA level increases in diseases result from the intracellular microorganisms as well. ADA is produced after alveolar macrophages are infected by M. tuberculosis, and is determined in serum during the active pulmonary tuberculosis. Lymphocytes, particularly T lymphocytes, have significant roles in the control of tuberculosis infection, in which lymphocytes turnover increases. The normalization of this turnover in any way result in decreased lymphocyte growth markers. Parallel to the decrease in leucocytes and lymphocytes, the level of serum ADA descents as well. Collazos et al have investigated the changes of serum ADA level during the pre-treatment and treatment period for 6 months. According to the result of the study, a progressive and significant decrease in serum ADA level was detected for the first 9 weeks of treatment. Perhaps this decrease might reflect the normalization of the altered lymphocyte turnover induced by tuberculosis [9].

Recently ADA levels have also been studied in sputum and serum for the diagnosis of tuberculosis, and ADA levels have been followed during the tuberculosis treatment [6-9, 22].

In a study by Kuyucu et al., the ADA level in the serum of children with tuberculosis was significantly higher than that of healthy children, and in tuberculoses diagnosis the cut-off value of serum ADA level was declared as 53,76U/l. Bhargave et al and Al-Shammary et al accepted the cut-off value of serum ADA level in tuberculosis patient as 78,12 and 32,8 respectively [6,8]. In the works undertaken by Conde MB et al., when 14 U/l is accepted as cut-off value for ADA level, in 49,2% of active tuberculosis patients this level was significantly higher than that of healthy individuals. In the study Collazos et al, the serum ADA level of 52% of active pulmonary tuberculosis patients was high. In another work undertaken by Yasuhara et al, ADA activity in children with active tuberculosis was higher than in children with bacterial or viral pneumonia. Alatas and et al. emphasized the importance of serum ADA levels in diagnosis and in following treatment answer [23]. In our study, serum ADA levels of all of pulmonary tuberculosis patients classified through diagnostic categories were higher than the values of healthy individuals, but the only statistically significant value belonged to the category I patients. This finding is likely to have resulted from the better responding cellular immunity in pulmonary tuberculosis patients who have received treatment and most likely to have been diagnosed for the first time than those who developed more or less resistance to tuberculosis drugs. Another finding supporting this idea is the fact that serum ADA level descends with the increasing number of resistant drug. Unfortunately the mean age of the patient and the control groups were statistically different in our study, so this restricted the results.

We determined significant difference between the ADA levels of the patients resistant to 3 and 4 drugs and patients

with no resistance to drugs. The ADA levels of the patients resistant to 3 and 4 drugs were significantly lower than the ADA levels of the patients with no resistance to drugs. The drug resistance is likely to be acquired, resulting from the long lasting pulmonary tuberculoses diagnosis and repeated anti-tuberculosis treatments. In conclusion we detected that Category I tuberculosis patients serum ADA levels were higher than control group and Category II tuberculosis patients although statistically not important, serum ADA levels were decreasing while the number of drugs that M. tuberculosis resistant increasing. But the mechanism that changing serum ADA levels with radiological widespread of tuberculosis and drug resistance should be searched in advanced studies.

Therefore, it is thought that in tuberculoses disease, immune mechanisms providing increased serum ADA levels may become insufficient and inefficient as the number of drugs the patients are resistant to increases.

While tuberculosis disease becomes chronic and treatment success decreased with drug resistance, serum ADA levels are decreasing or the mechanism that increases serum ADA levels failed and treatment become harder.

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