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Original Article

Diagnostic Value of Serum Adenosine Deaminase Level in Extrapulmonary Tuberculosis

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Abstract

Background: Although tuberculosis is a known disease, still on occasion, the tuberculosis patients remain undiagnosed and hence not treated appropriately. In some cases, the patient is not able to give a proper sputum sample. In some other cases, expert laboratory technician to examine the patient's sputum smear is not available. Furthermore, and in most cases of extrapulmonary tuberculosis, invasive diagnostic procedures such as biopsy might be followed. The aim of this study was to investigate the diagnostic value of serum level of adenosine deaminase (ADA) in extra pulmonary tuberculosis.

Methods: In this analytical study, pulmonary tuberculosis patients were compared with two other groups of patients including lung cancer patients and healthy controls, based on national tuberculosis protocol. In each group, 40 patients who almost matched for age and sex were included. Blood samples were taken from the participants to measure serum ADA levels. Data were analyzed using SPSS software version 15.0.

Results: Based on the data analyzed in this study, the mean ADA level in the patients with extra pulmonary tuberculosis was 23.8 IU/L which was significantly higher than that in the other groups ($P<0.001$). Mean ADA levels in lung cancer patients and in healthy subjects were 15.8 IU/L and 10.7 IU/L, respectively.

Conclusions: According to the results of this study, higher cut-off value for ADA would increase the specificity up to 100%. Moreover, serum ADA level can be a valuable additional index in diagnosis of extra pulmonary tuberculosis.

Keywords: Pulmonary tuberculosis, Lung cancer, Adenosine deaminase

Background

Tuberculosis (TB) is an infectious disease with a known etiologic agent and epidemiological pattern, and World Health Organization (WHO) has recommended its treatment about 60 years ago (1,2). Although Directly Observed Treatment Short course (DOTS) strategy has been used for more than a quarter of a century to control it, still a considerable number of the TB patients in many parts of the world, including Iran are left undiagnosed and not treated appropriately (1,3,4). Therefore, TB remains a major health problem in many parts of the world (1,3,5). Almost one third of the world population (around 2 billion people) are infected with TB and are at risk to develop active pulmonary TB (1,3,6). Every year about 9 million people develop active TB and 1.5 to 2 million people die of the disease (1). Over 90% of TB cases and deaths occur in developing countries, where 75% are in most economically active age group (15 to 54 years) (1). According to published studies, TB is an important public health problem in Khuzestan, south west of Iran (5,7,8). Diagnosis of TB is not always so easy. In some cases, the patient is not able to give a proper sputum sample, and in some other cases, an experienced health care worker who can carefully examine the sputum smear is not available (2,7). In cases of extra pulmonary TB, in most cases there is a need for invasive diagnostic procedures such as biopsy. Although the standard method for diagnosis of TB is isolation of *Mycobacterium tuberculosis* from the specific culture media, in the above-mentioned condition, a diagnostic test with a shorter period of time and acceptable sensitivity and specificity is necessary to detect the disease (1,2,7). The enzymatic activity of adenosine deaminizes (ADA, adenosine amino hydrolase E.C. 3.5.4.4) in serum and body fluids such as pleural effusion, ascites and cerebrospinal fluid has been taken into consideration (2,9-12). ADA enzyme plays a leading role in the metabolism of purines. It also catalyzes hydrolytic deamination of adenosine to inosine as well as

deoxyadenosine to deoxyinosine. Moreover, this enzyme plays a remarkable physiological role in regulating the effect of these metabolites on immunological processes, and neurological and vascular proliferation and differentiation of lymphocytes, particularly T lymphocytes (2,9-12). The ADA level in lymphocytes is ten times more than that in red blood cells. Additionally, the ADA level in B lymphocytes is more than that in T lymphocytes (13). The lack of the enzyme in the body is associated with severe cellular and humoral immune deficiencies. Increased serum ADA activity can be seen in many of the diseases , including typhoid fever, infectious mononucleosis, liver disease, sarcoidosis, leukemia, brucellosis, acute pneumonia, rheumatoid arthritis, malignancy, and TB that are associated with stimulating the cellular system (1,12). Increased serum ADA activity has also been reported in infants after BCG vaccination (11).

With the above-mentioned problems regarding the diagnosis of TB in mind, in this context we tried to investigate the diagnostic value of serum ADA level in extra pulmonary TB (5,11). Numerous study results in confirming the diagnostic value of ADA are required, the evaluation of which may contribute to the suggestion of this method as a reliable strategy in the diagnosis of extra pulmonary TB (2,5). We designed this study as similar surveys in other parts of the world got different results. If a study could confirm the diagnostic value of this method, it could help us in early diagnosis of extra pulmonary TB, and accordingly a remarkable decrease in morbidity and mortality rate could be achieved.

Objectives

The aim of this study was to investigate the diagnostic value of serum ADA level in extra pulmonary TB.

Methods

Study Design

This cross-sectional study was carried out in Ahvaz (Southwest of Iran) in 2016. A total of 40 patients with pathology-, smear-, and culture-based diagnosis of extra pulmonary TB were enrolled in this study. Exclusion criteria included: patients who had lung cancer in addition to TB, those with other respiratory infections, or the patients immunocompromised for any reason (congenital or acquired). In this study, two other groups (40 people in each), matched for age and sex, were also included. One group included 40 patients with malignancies and the other group had 40 healthy individuals in every respect. After obtaining informed consent and filling out the questionnaire for all three groups, 3 mL of blood sample were collected in aseptic conditions from each participant and immediately sent to the laboratory to separate the serum. In the laboratory, ADA levels were measured by the method of Galanti and Guisti. Serum ADA activity was expressed as IU/L. Each 1 IU / L ADA was defined as one mole ammonia produced in one minute in a liter of serum.

Statistical Analysis

In this study, SPSS software version 15.0 was used for data analysis. Chi-square test was used for qualitative variables. To determine the diagnostic value of serum ADA activity, the ROC (receiver operator characteristic) curve method was used. ANOVA was also used to compare the groups for quantitative variables. Normality of data was tested by sample Kolmogorov-Smirnov test. In this test, the *P* value greater than 0.05 contributes to normal distribution of data. The homogeneity of the data was tested using homogeneity of variances or the Levene's test. In this test, the *P* value greater than 0.05 indicates the homogeneity of data. In case of non-homogeneity of the groups when using ANOVA test, post hoc test could be chosen where part of the group is heterogeneous; and one of the most famous cases of these tests is Dunnett's test.

Results

Despite the fact that the patients were matched for the age and sex, since the patients with lung cancer were mostly older than those with TB, therefore two groups disagreed in age. In the TB group 15 males and 25 females, in the cancer group 19 males and 21 females, and in the control group 21 males and 19 females participated. Age groups are shown in Figures 1.

The distribution of data was normal in all the study groups (*P* value was 0.2, 0.221, and 0.111 for the control, TB, and lung cancer groups, respectively) (Table 1). Homogeneity of variances test showed that the data were not homogeneous ($P<0.001$). Moreover, the results of ANOVA test showed a significant difference between the groups ($P<0.001$). ANOVA results are shown in Table 2.

Considering the non-homogeneity of the groups, they were compared using the Dunnett's test (Table 3).

Furthermore, Pearson correlation coefficient was conducted between the age and the ADA level in each

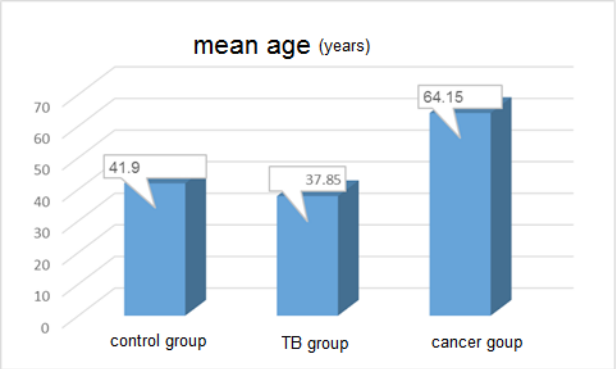

Figure 1. Mean Age of the Patients in the Control, TB, and Lung Cancer Groups.

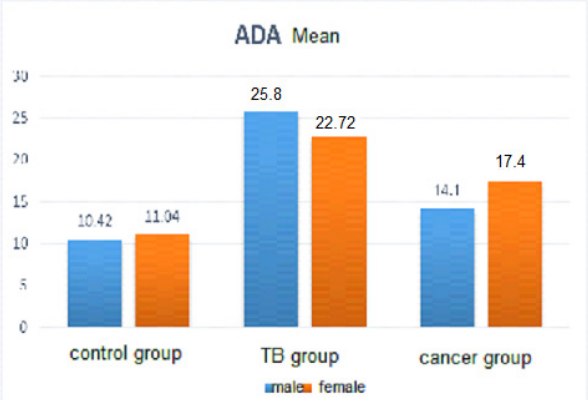

Figure 2. Mean Serum ADA Levels in the Study Groups Arranged Based on Sex

Table 1. Comparison of Mean Serum Levels of ADA Among the Study Groups

Groups	Descriptive				
	Mean	Variance	SD	Min	Max
Control	10.75	7.57	2.75	5	15
TB	23.875	99.18	9.95	13	51
Cancer	15.875	85.34	9.23	5	5

Table 2. ANOVA Test Among the Study Groups

ANOVA	Changes	*df*	Mean Square	Test	*P* Value
Intergroups	3527.82	2	1763.91		
Within groups	7315.56	116	63.06	27.97	0.000
Total	10843.39	118	-		

Table 3. Differences Between the Groups Using Dunnett's Test

Groups	Mean Difference	*P* Value
TB and control groups	-13.12	0.000[a]
Cancer and control groups	-4.78	0.009[a]
TB and cancer groups	8.33	0.001[a]

[a] Significant.

group. The results are shown in Table 4.

As shown in Table 4, no significant correlation was found between age and the ADA level in none of the groups. The sensitivity and specificity of statistical analysis was tested using ROC curve method for TB (results are shown in Table 4 and Figure 3). According to the curve coordinates, If ADA cutoff point was equal to 13, 97% sensitivity and 88% specificity could be observed, and if it was equal to 20, the sensitivity and specificity would be 55% and 100%, respectively.

Discussion

Worldwide studies have revealed that the diagnostic value of ADA in extra pulmonary TB has been associated

Table 4. The Correlation Coefficient Between Age and the ADA Level Among the Study Groups

Age	Correlation	*P* Value
Control group	0.074	0.652
TB group	-0.06	0.713
Cancer group	-0.2	0.216

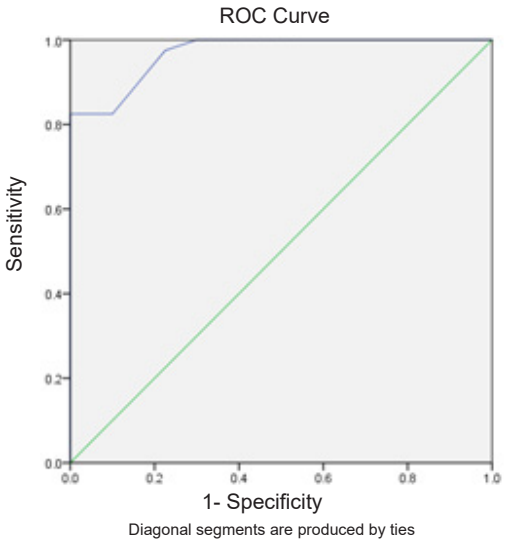

Figure 3. Sensitivity and Specificity of ADA in TB Diagnosis.

with controversial results (6-10). The present study demonstrated that the measured levels of ADA could be an acceptable contributing index in the diagnosis of extra pulmonary TB. In this study, mean levels of ADA in the patients with extra pulmonary TB were significantly higher than that in the other groups. Searching the medical literature for the diagnostic value of serum ADA level in extra pulmonary TB got to no result. Most published studies were conducted on the ADA level in liquids such as pleural effusion, cerebrospinal fluid, and peritoneal fluid (9-12). In a prospective study by Khan et al, during 2009-2010, on the value of ADA and IFN- γ in pleural fluid for the diagnosis of TB pleurisy, IFN-γ and ADA could be used as valuable parameters for the differentiation of TB from nontuberculous effusion, and IFN-γ was more sensitive and specific than ADA for TB effusion. The results for IFN-γ versus ADA were: sensitivity, 100% versus 86%, respectively; specificity, 100% versus 74%, respectively; positive predictive value, 100% versus 88.5%, respectively; and negative predictive value, 100% versus 69.7%, respectively (14). Valdes et al conducted a study on the patients hospitalized due to pleural effusion in Spain. The patients were divided into three groups: patients with definitive diagnosis of TB, malignancy, and para pneumonic. ADA, lysozyme, and IFN-γ levels were measured in pleural fluid and compared with those levels in serum, respectively. ADA was equal

to or greater than 47 U/L in 100% of patients with TB pleurisy, but only in 5% of the patients with non-TB pleurisy (sensitivity 100% and specificity 89%). An ADA ratio more than 1.5 for pleural fluid to serum was seen in 85.7% of the patients with TB pleurisy (sensitivity 85.7%, specificity 89%) (15). A study by Saleh et al on the patients with TB peritonitis in Egypt showed that ADA levels ≥ 35 IU/L in peritoneal fluid with a sensitivity of 100% and specificity of 93.5% is a suitable method for the diagnosis of TB peritonitis (16). The study of Abdolbagi and colleagues in Guilan on meningitis patients (in two groups: definitely diagnosed with TB meningitis, and patients with meningitis caused by other causes) showed that in the first group ADA level in the cerebrospinal fluid (20.8 IU/L) was significantly higher than that in non-TB meningitis (9.75 IU/L). ADA enzyme activity above the cutoff point of 11 IU/L achieved sensitivity of 73% and specificity of 66% in the diagnosis of TB meningitis. They showed that ADA measurement alone is not helpful in the diagnosis, and consideration of other factors is needed for quick diagnosis (17). In a study on patients with cerussite (pleurisy, peritonitis, and pericarditis) in India, Mathur and colleagues showed that ADA levels in inflammatory liquid is a useful index in the diagnosis of TB cerussites with a sensitivity of 96.4% and specificity of 100% (18). Tuon et al in a systematic review on the articles published between 1966 and 2007 in Sao Paulo, Brazil, on the value of the ADA level in the cerebrospinal fluid for the diagnosis of TB meningitis, showed that this method does not have sufficient sensitivity and specificity in differentiating TB meningitis from non-TB (19,20).

Limitations and Strengths

Not surprisingly, there are some limitations in this study, from which the short time span (one year) on which the study was conducted and the number of studied cities (one city) could be referred to. In this regard, conducting similar studies in Khuzestan province and overall in Iran is recommended.

Conclusions

Based on the results of this study, measured levels of ADA is one of the most important indexes in the diagnosis of extra pulmonary TB. In our search of the literature on the diagnostic value of serum ADA levels for extra pulmonary TB, except for one paper, no other studies were found. Therefore, before recommending the use of this method for the diagnosis of extra pulmonary TB, more studies are required to be conducted on a larger number of patients.

Conflict of Interests

Authors declare no conflict of interests associated with this study.

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References

1. Fitzgerald D, Haas DW. Mycobacterium tuberculosis. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases. 6th ed. Philadelphia: Churchill Livingstone; 2005:2852-2886.
2. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic Value of Serum Adenosine Deaminase (ADA) Level for Pulmonary Tuberculosis. Jundishapur J Microbiol. 2015;8(3):e21760. doi: 10.5812/jjm.21760.
3. World Health Organization (WHO). Global status report on road safety: time for action. Geneva: WHO; 2009.
4. Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia A, Garcia-Ferrer D, Iborra-Millet J, Ferrero-Vega JA, et al. Tuberculosis and Vitamin D Status Among the Contacts of Pulmonary Tuberculosis Patients. Avicenna J Clin Microbiol Infect. 2017;4(1):e36889. doi: 10.17795/ajcmi-36889.
5. Alavi SM, Sefidgaran GH. Tuberculin survey among school-aged children in Ahvaz, Iran, 2006. Int J Infect Dis. 2008;12(4):406-9. doi: 10.1016/j.ijid.2007.11.005.
6. Hashemi SH, Mamani M, Alizadeh N, Nazari M, Sedighi I. Prevalence of tuberculosis infection among health-care workers in Hamadan, west of Iran. Avicenna J Clin Microbiol Infect. 2014;1(1):e19214. doi: 10.17795/ajcmi-19214.
7. Alavi SM, Khoshkhoy MM. Pulmonary tuberculosis and diabetes mellitus: Co-existence of both diseases in patients admitted in a teaching hospital in the southwest of Iran. Caspian J Intern Med. 2012;3(2):421-4.
8. Alavi SM, Salami N. The causes of death among patients with tuberculosis in Khuzestan, Iran. Pak J Med Sci. 2008;24(2):217-20.
9. Afrasiabian S, Mohsenpour B, Bagheri KH, Sigari N, Aftabi K. Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis. J Res Med Sci. 2013;18(3):252-4.
10. Dimakou K, Hillas G, Bakakos P. Adenosine deaminase activity and its isoenzymes in the sputum of patients with pulmonary tuberculosis. Int J Tuberc Lung Dis. 2009;13(6):744-8.
11. Kartaloglu Z, Okutan O, Bozkanat E, Ugan MH, Ilvan A. The course of serum adenosine deaminase levels in patients with pulmonary tuberculosis. Med Sci Monit. 2006;12(11):Cr476-80.
12. Lamsal M, Gautam N, Bhatta N, Majhi S, Baral N, Bhattacharya SK. Diagnostic utility of adenosine deaminase (ADA) activity in pleural fluid and serum of tuberculous and non-tuberculous respiratory disease patients. Southeast Asian J Trop Med Public Health. 2007;38(2):363-9.
13. Russo M, Giancane R, Apice G, Galanti B. Adenosine deaminase and purine nucleoside phosphorylase activities in peripheral lymphocytes from patients with solid tumours. Br J Cancer. 1981;43(2):196-200.
14. Khan FY, Hamza M, Omran AH, Saleh M, Lingawi M, Alnaqdy A, et al. Diagnostic value of pleural fluid interferon-gamma and adenosine deaminase in patients with pleural tuberculosis in Qatar. Int J Gen Med. 2013;6:13-8. doi: 10.2147/ijgm.s39345.
15. Valdes L, San Jose E, Alvarez D, Sarandeses A, Pose A, Chomon B, et al. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. Chest. 1993;103(2):458-65.
16. Saleh MA, Hammad E, Ramadan MM, Abd El-Rahman A, Enein AF. Use of adenosine deaminase measurements and

QuantiFERON in the rapid diagnosis of tuberculous peritonitis. J Med Microbiol. 2012;61(Pt 4):514-9. doi: 10.1099/jmm.0.035121-0.
17. Abdolbagi MH, Dezfuli AR, Soudbakhsh AR, Rasulinejad M, Golestani B. Diagnostic evaluation of adenosine deaminase in cerebrospinal fluid in tuberculous meningitis. Kanun Med J. 2006;8(4):23-8.
18. Mathur PC, Tiwari KK, Trikha S, Tiwari D. Diagnostic value of adenosine deaminase (ADA) activity in tubercular serositis. Indian J Tuberc. 2006;53(2):92-5.
19. Tuon FF, Higashino HR, Lopes MI, Litvoc MN, Atomiya AN, Antonangelo L, et al. Adenosine deaminase and tuberculous meningitis--a systematic review with meta-analysis. Scand J Infect Dis. 2010;42(3):198-207. doi: 10.3109/00365540903428158.
20. al-Shammary FJ. Adenosine deaminase activity in serum and pleural effusions of tuberculous and non-tuberculous patients. Biochem Mol Biol Int. 1997;43(4):763-79.