Diagnostic value of sputum adenosine deaminase (ADA) level in pulmonary tuberculosis

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Abstract

Introduction **Tuberculosis is still a considerable health problem in many countries. Rapid diagnosis of this disease is important, and adenosine deaminase (ADA) has been used as a diagnostic test. The aim of this study was to assess the diagnostic value of ADA in the sputum of patients with pulmonary tuberculosis.**

Methods **The current study included 40 patients with pulmonary tuberculosis (culture positive, smear ±) and 42 patients with non tuberculosis pulmonary diseases (culture negative). ADA was measured on all of the samples.**

Results **The median value of ADA in non-tuberculosis patients was 2.94 (4.2) U/L and 4.01 (6.54) U/L in tuberculosis patients, but this difference was not statistically significant (p=0.100). The cut-off point of 3.1 U/L had a sensitivity of 61% and a specificity of 53%, the cut-off point of 2.81 U/L had a sensitivity of 64% and a specificity of 50% and the cut-off point of 2.78 U/L had a sensitivity of 65% and a specificity of 48%. The positive predictive values for cut-off points of 3.1, 2.81 and 2.78 U/L were 55.7%, 57.44% and 69.23%, respectively. The negative predictive values for the abovementioned cut-off points were 56.75%, 57.14% and 55.88%, respectively.**

Conclusion **Our results showed that sputum ADA test is neither specific nor sensitive. Because of its low sensitivity and specificity, determination of sputum ADA for the diagnosis of pulmonary tuberculosis is not recommended.**

Keywords **Tuberculosis, diagnosis, ADA, sputum**

Introduction

One hundred years after the discovery of tuberculosis bacillus, the disease is still one of the considerable health problems in many human societies. In general, 8.5 million people have been stricken by tuberculosis (TB) each year and 1.3 million of them died (including 320,000 deaths among people who are HIV positive).[1] According to the last record of WHO, Iran has the lowest incidence rate of tuberculosis among the neighbors.[2] Rapid diagnosis of symptomatic disease is a base for proper disease management, and the gold standard test for diagnosis is mycobacterial culture. However, the growth of tubercle bacilli in culture medium requires six weeks and the determination of sensitivity to drugs also needs three to six more weeks,[3] while semi-automated and fully-automated continuous monitoring systems for growth and detection of mycobacteria are commercially available. With each system, bottles are incubated in the specific instrument, where they are monitored for changes in production or production and consumption of various gases, indicating growth

Received: 04 January 2016; revised 10 February 2016; accepted: 18 February 2016

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Published June 2016

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

of mycobacteria. However, these systems are expensive and not available in every laboratory.[4] Other diagnostic methods have their own difficulties, for example the sensitivity of Ziehl-Neelsen stain is 10% to 40%. Polymerase chain reaction (PCR) is a rapid, sensitive and specific method for the diagnosis of tuberculosis and the result is generally available in a day or two. PCR has a high specificity (78% to 100%) but according to the method which is used, its sensitivity differs from 20% to 90%. The sensitivity of this test depends on the type of primer used, the sequence of the multiplied genome, the number of mycobacteria and the existence of proliferation inhibitors.[5] In addition, this method is not available in every laboratory. Consequently, application of a rapid, cheap and available test seems necessary. Since 1978, adenosine deaminase (ADA) has been used as a diagnostic test in tuberculous effusions.[6] ADA plays an important role in the process of lymphocyte differentiation[7] as ADA is a molecular product of purine which contains a sugar molecule, namely ribose. This enzyme irreversibly converts adenosine to inosine and deoxyadenosine to deoxyinosine, and the speed rate of this reaction gives an account of enzymatic activity, calculated as ADA concentration in the laboratory.[8] A meta-analysis of studies done from 1966 to 1999 displayed that the efficiency of the ADA test in diagnosing tuberculous pleural effusion was acceptable and it was reported that its sensitivity varied from 47.1% to 100% while its specificity ranged from 0% to 100%.[5] This meta-analysis is representative of the diversity of results among the published studies. On the other hand results of a systematic review which was done in 2007 showed that there is no evidence on the usefulness of ADA in the diagnosis of pulmonary tuberculosis.[9] In addition, the reported ADA cutoffs are often derived from studies based on different methods of ADA evaluation. Although a review article has confirmed the sensitivity and specificity of ADA in the diagnosis of tuberculosis of the pericardium, central nervous system, peritoneum and pleura,[3] there are few studies on the value of the measurement of ADA in obtained samples using non-invasive methods such as sputum.[10-13] In the current study, we have assessed the diagnostic value of ADA in sputum in patients with suspected pulmonary tuberculosis.

Methods

We performed a cross-sectional study on the patient population of the Nikoopoor Health Center, Yazd, Iran. Briefly, the inclusion criteria selected all patients suspected to have pulmonary tuberculosis and who had received no treatment. Eighty two patients suspected of pulmonary tuberculosis were transferred to the Nikoopoor Health Center along with their sputum samples (tuberculosis reference laboratory), and were divided into two groups – patients with pulmonary tuberculosis (smear-positive and culture-positive, or smear-negative and culture-positive) and patients with non-tuberculosis pulmonary diseases (smear-negative and culture-negative). The sputum was naturally expectorated in all of the patients. As a result, contamination with saliva and/or nasal secretions was similar among the two groups. Egg-based (Löwenstein-Jensen) solid medium was used for mycobacterial culture. All cultures were incubated at 37°C in an atmosphere of 5-10% carbon dioxide (CO_2) and examined weekly for at least 6 weeks. Meanwhile, an ADA enzyme test was done on all of the samples at the time of reception regardless of smear and culture response, to compare the groups considering ADA enzyme activity. To do this test (Diazyme, Poway, CA, USA), sputum was initially homogenized with 70 millimol phosphate buffer (pH: 6.0) containing 0.5 mol NaCl and then stored at a temperature of 4°C for 12 hours and centrifuged at 16,000 rpm for 30 min at a temperature of -4°C. Finally, the samples were all kept at -70°C to be analyzed. After getting the results of ADA, the statistical significance of differences between median values was estimated by Kruskal-Wallis and Mann-Whitney U tests, as the distribution of the variables was abnormal. Receiver operating characteristic (ROC) curve was generated for ADA values and its accuracy was measured by the area under the ROC curve. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS.16 (SPSS Inc., Chicago, IL, USA).

The research was based on Helsinki declaration ethical principles and written informed consent was obtained from all participants.

Results

The study included 40 patients with pulmonary tuberculosis (culture-positive, smear ±) and 42 patients with non-tuberculosis pulmonary diseases (smear-negative, culture-negative). The patients were stratified into 4 groups based on age, ≤30 (16 patients), 31-45 (11 patients), 46-60 (21 patients), >60 (34 patients); 33 patients were women (40.2%) and 49 patients were men (59.8%). Of all participants in this study, 62 patients (75.6%) were Iranian and 20 patients (24.4%) were non-Iranian. Despite negative culture, 4 patients received anti-tuberculosis therapy, owing to their clinical symptoms. Their ADA values were 3.8, 2.78, 2.65 and 2.75 U/L, respectively.

The median value of ADA did not differ statistically between tuberculosis and non-tuberculosis patients (p=0.100), nor between genders (p=0.500) and age (p=0.200, Table 1). However, the median value of ADA in non-Iranian patients was significantly higher than in Iranian patients (p=0.006).

When ascertaining the optimum cut-off points of the ROC curve (Table 2, Figure 1), the cut-off point of 3.1 U/L had a sensitivity of 61% and a specificity of 53%, the cut-off point of 2.81 U/L had a sensitivity of 64% and a specificity of 50% and the cut-off point of 2.78 U/L had a sensitivity of 65% and a specificity of 48%. The positive predictive value (PPV) for cut-off points of 3.1, 2.81 and 2.78 U/L was 55.7%, 57.44% and 69.23%, respectively. The negative predictive value (NPV) for the above-mentioned cut-off points was 56.75%, 57.14% and 55.88%, respectively. The accuracy of the ROC curve in diagnosing tuberculosis and non-tuberculosis cases was measured by the area under the curve, which was 59%, non-suggestive for accuracy (Figure 1).

Table 1. Comparison of median ADA value according to study group, gender, age and race

Characteristics		Median ADA value (U/L)	IQR	P-value
Study group	TB (n=42)	4.01	6.54	0.100
	Non-TB (n=40)	2.94	4.2	
Gender	Female	2.78	5.29	0.500
	Male	3.96	5.08	
Age	<30	4.56	8.07	0.200
	31-45	2.20	2.48	
	46-60	3.16	9.78	
	>60	3.33	4.29	
Race	Iranian	2.96	3.76	0.006
	Afghans	6.21	13.9	

ADA – adenosine deaminase; **IQR** – interquartile range; **TB** – tuberculosis

Table 2. Sensitivity and specificity of sputum ADA cut-off points

Cut-off point (U/L)	Sensitivity	Specificity
3.1	61%	53%
2.81	64%	50%
2.78	65%	48%

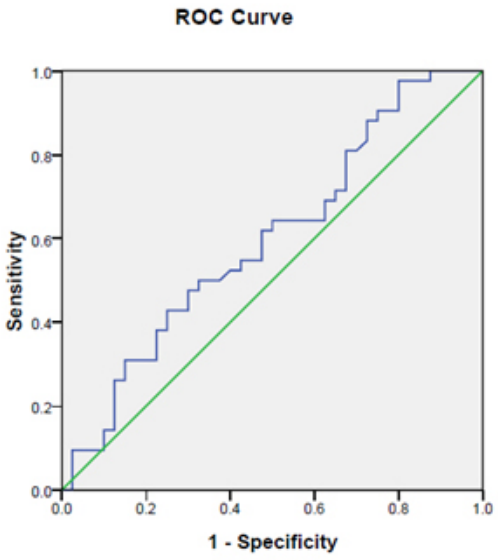

Figure 1. Receiver operating characteristic curve for the ability of sputum ADA to differentiate patients with pulmonary tuberculosis

Discussion

Our results showed that ADA values in the sputum of patients suffering from pulmonary TB were higher than in non-tuberculosis patients,

however, this difference was not meaningful. Normally, the ADA level in macrophages and monocytes is low, except when these cells are triggered by the infection with intracellular microorganisms, and it has been shown that this increase is related to the rise in ADA-2 level.[14] A review article has confirmed the sensitivity and specificity of ADA in diagnosing tuberculosis of the pericardium, central nervous system, peritoneum and pleura.[3] Measuring the ADA level is currently considered the gold standard in developing countries for diagnosing pleural tuberculosis.[15] One study showed that serum ADA levels are significantly higher in TB patients compared with controls.[16] The outcomes of the study conducted by Alatas et al. indicated that ADA levels in pulmonary TB patients were higher than in the control population, and that ADA levels in patients with tuberculosis pleurisy were higher than in patients with other tuberculosis manifestations.[17] In a study that investigated extrapulmonary TB cases, there was a statistically meaningful distinction in the ADA level in tuberculosis and non-tuberculosis disease, and the ADA level in effusions had a considerable value, accuracy, and sensitivity in differentiating tuberculosis cases from non-tuberculosis cases. Therefore in TB endemic countries, patients with raised ADA levels could be considered to have tuberculosis where acid-fast bacilli positivity is low.[18] The results of some studies have shown that measuring ADA levels in serum and bronchoalveolar lavage fluid can be effective in differentiating between pneumonia, pulmonary TB and lung cancer.[19,20] However, few studies have investigated the diagnostic value of ADA in the sputum. In fact Dimakou et al.[11] were among the first to show that the value of ADA in sputum in patients suffering from TB was higher than in patients suffering from lung cancer, both in individuals with positive and negative smear. Again in 2009 Dimakou[12] showed that the ADA level in the sputum of tuberculosis patients was higher than in patients suffering from lung cancer. But another study which was conducted by Dilmac indicated that, because of a low sensitivity, the routine measurement of ADA in sputum was not appropriate in the diagnosis of pulmonary TB, but that it can be effective in diagnosing patients who strongly are suspected to suffer from TB and who had negative smear.[10] Another study showed that the ADA level in the sputum of the patients suffering from TB was significantly higher than in patients with lung cancer (particularly ADA-2).[13] In another study the average sputum ADA and alkaline phosphatase activity were significantly higher in pulmonary TB in comparison with lung cancer, and authors concluded that this test could be useful in diagnosing pulmonary TB.[7] In a recent study sputum ADA appeared to be more sensitive compared with serum ADA in the diagnosis of pulmonary tuberculosis, with a statistically significant positive correlation between sputum and serum ADA.[21]

In the present study the ADA values in the sputum of patients suffering from pulmonary TB were higher than in non-tuberculosis patients, but this difference was not meaningful. The reason for this difference between the present study and other studies can be related to the difference in measuring ADA, TB epidemiology, race and dissimilarities in control groups. Two main ways for measuring ADA are Giusti and non-Giusti. In the method of Giusti[22] ammonia is measured with Berthelot's reaction which is not easily automated. Diazyme however is one of the non Giusti-methods. Song et al. noted a significant difference in ADA levels between different methods.[23] In the present study the Diazyme method was used, which is a non-Giusti method while in other studies the Giusti method was used. It is also important to evaluate new methods for measuring ADA in comparison with clinical outcomes. On the other hand it has been shown that using the various ADA isoenzymes and the isoenzyme ratio can be effective in the segregation of different reasons of increasing ADA in body fluids. In our study we were not able to measure ADA isoenzymes, because the kit was not available at that time in our city. In addition the extraction of ADA from the sputum is difficult and high levels of viscid DNA and proteases from various inflammatory cells in the sputum can have an impact on the assay.[24]

Determining the cut-off point in the obtained sensitivity and specificity in different studies is effective. A meta-analysis[5] found that the reported cut-off point in different studies was between 30 and 71 U/L. According to the cut-off point which has been used in different researches, sensitivity, specificity, PPV, and NPV have been reported differently, and therefore the outcomes of different studies must be interpreted cautiously. Since there is no program in order to standardize the ADA results, determining a cut-off point for ADA must be dependent on the type of method and defined separately for each area. TB epidemiology is another important factor. Studies have shown that in areas in which tuberculosis is endemic, the test sensitivity is of high importance.[25] In this area the best way for quick diagnosis of tuberculosis is ADA measurement.[26] However, although the ADA sensitivity is high, its specificity is low, because ADA increases in other diseases such as lung cancer, lymphoproliferative disorders, empyema, mesothelioma, and rheumatologic diseases.[27] In areas in which the prevalence of tuberculosis disease is low, PPV values decrease, and as a result it is highly probable to obtain false positive results using this test. It seems that racial differences also have an impact on the obtained values in various studies, and in our study the ADA level in the sputum of migrants (Afghans) was higher than in domestic patients. An important fact in this study is that exact and careful criteria have been used in such a way that only patients who had positive culture were considered as pulmonary TB patients, while in other studies TB diagnosis is clinical and as we know the ADA level can increase in other pulmonary diseases. This matter can cause some disagreements between the present outcomes and other studies.

Conclusion

In conclusion our results showed that the sputum ADA test is neither specific nor sensitive. Reproducibility and accuracy of ADA measurement in the sputum have not been investigated widely; as a result its use as clinical instrument needs verification. Because of its low sensitivity and specificity, determination of ADA level in sputum for the diagnosis of pulmonary TB is not recommended.

Acknowledgment: The authors would like to thank the Yazd Shahid Sadoughi University of Medical Sciences. This study was funded by a grant from the Deputy for Research of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The research was also a thesis presented for obtaining the specialty of Pathology degree by Hadi Jalali, MD.

Authors' contributions statement: Authors had equal contributions. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

References

1. World Health Organization. Global tuberculosis report 2013. WHO, Geneva; 2013. Accessed on 10 Sep 2015. Available at: http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf
2. World Health Organization. Global health observatory data repository. Accessed on: 25 Dec 2015. Available at: http://apps.who.int/gho/data/node.main.1316
3. Barua R, Hossain M. Adenosine deaminase in diagnosis of tuberculosis: a review. Anwer Khan Mod Med Coll J 2014;5:43-8. [CrossRef]
4. Pincus MR, Henry JB, McPherson RA. Henry's clinical diagnosis and management by laboratory methods: Elsevier Saunders; 2011.
5. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T. Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Ann Clin Biochem 2003;40:374-81. [CrossRef] [PubMed]
6. Piras MA, Gakis C, Budroni M, Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br Med J 1978;2:1751-2. [CrossRef] [PubMed] [FullText]
7. Jadhav AA, Jain A. Sputum adenosine deaminase and alkaline phosphatase activity in pulmonary tuberculosis. Arch Physiol Biochem 2012;118:6-9. [CrossRef] [PubMed]
8. Verma M, Narang S, Moonat A, Verma A. Study of adenosine deaminase activity in pulmonary tuberculosis and other common respiratory diseases. Indian J Clin Biochem 2004;19:129-31. [CrossRef] [PubMed] [FullText]
9. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007;11:1-196. [CrossRef] [PubMed]
10. Dilmaç A, Uçoluk GO, Uğurman F, et al. The diagnostic value of adenosine deaminase activity in sputum in pulmonary tuberculosis. Respir Med 2002;96:632-4. [CrossRef] [PubMed]
11. Dimakou K, Bakakos P, Samara I. Adenosine deaminase activity in sputum from patients with pulmonary tuberculosis. Eur Resp J 1997;10:450s.

12. 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;13:744-8. [PubMed]
13. Samara I, Bakakos P, Orphanidou D, et al. Sputum adenosine deaminase activity in patients with pulmonary tuberculosis and lung cancer. Adv Clin Exp Med 2007;16:533.
14. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. Eur Respir J 1996;9:632-3. [CrossRef] [PubMed]
15. Corral I, Quereda C, Navas E, et al. Adenosine deaminase activity in cerebrospinal fluid of HIV-infected patients: limited value for diagnosis of tuberculous meningitis. Eur J Clin Microbiol Infect Dis 2004;23:471-6. [CrossRef] [PubMed]
16. Boloursaz M, Khalilzadeh S, Khodayari A, Hakimi S. Adenosine deaminase level as an indicator for differentiating between active pulmonary tuberculosis infection and other pulmonary infections. J Compr Pediatr 2012;3:3-6. [CrossRef]
17. Alataş F, Uslu S, Moral H, et al. [Serum adenosine deaminase activity in pulmonary tuberculosis]. Tuberk Toraks 2003;51:277-81. [PubMed]
18. Gupta BK, Bharat V, Bandyopadhyay D. Sensitivity, specificity, negative and positive predictive values of adenosine deaminase in patients of tubercular and non-tubercular serosal effusion in India. J Clin Med Res 2010;2:121-6. [CrossRef] [PubMed] [FullText]
19. Hassanein K, Hosny H, Mohamed R, Abd El-Moneim W. Role of adenosine deaminase (ADA) in the diagnosis of pulmonary tuberculosis. Egypt J Bronchol 2010;4:11-8.
20. Kayacan O, Karnak D, Delibalta M, Beder S, Karaca L, Tutkak H. Adenosine deaminase activity in bronchoalveolar lavage in Turkish patients with smear negative pulmonary tuberculosis. Respir Med 2002;96:536-41. [CrossRef] [PubMed]
21. Atta S, Kassem A, Elhadidi A, El Esawy H. The diagnostic value of adenosine deaminase activity in pulmonary tuberculosis: Comparison between sputum and serum. Egypt J Chest Dis Tuberc 2015;64:103-7. [CrossRef]
22. Giusti G, Galanti B. Colorimetric method. Methods of enzymatic analysis. Weinheim: Verlag Chemie 1984:315-23.
23. Song D, Lun AR, Chiu W. Diazyme adenosine deaminase in the diagnosis of tuberculous pleural effusion: method evaluation and clinical experiences in a New Zealand population. N Z J Med Lab Sci 2010;64:11.
24. Kelly MM, Keatings V, Leigh R, et al. Analysis of fluid-phase mediators. Eur Respir J Suppl 2002;37:24s-39s. [PubMed]
25. Mohammadtaheri Z, Mashayekhpour S, Mohammadi F, Mansoori D, Masjedi MR. Diagnostic value of adenosine deaminase isoenzyme (ADA2) and total ADA in tuberculous pleural effusion. Tanaffos 2005;4:37-42.
26. Valdés L, Alvarez D, San José E, et al. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. Thorax 1995;50:600-3. [CrossRef] [PubMed] [FullText]
27. Valdés L, San José E, Alvarez D, Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy. Eur Respir J 1996;9:747-51. [CrossRef] [PubMed]

Please cite this article as:
Binesh F, Jalali H, Zare MR, Behravan F, Tafti AD, Behnaz F, Tabatabaee M, Shahcheraghi SH. Diagnostic value of sputum adenosine deaminase (ADA) level in pulmonary tuberculosis. GERMS 2016;6(2):60-65. doi: 10.11599/germs.2016.1090