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Adenosine Deaminase Activity in Bronchoalveolar Lavage Fluid in Patients with Smear-Negative Pulmonary Tuberculosis

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ABSTRACT

Background: Tuberculosis (TB) remains a major health problem across the world and most commonly involves the lungs. Diagnosis of TB is based on finding acid-fast bacilli (AFB) in sputum or a positive sputum culture. The sensitivity of sputum smear is only 40-70% and it takes 4-8 weeks for sputum culture results. We decided to measure adenosine deaminase (ADA) activity in bronchoalveolar lavage (BAL) fluid and compare it with sputum and BAL fluid cultures.

Materials and Methods: A descriptive study was performed at the Shahid Sadoughi Hospital in Yazd, from 2005 to 2006. Sixty-three patients suspected for pulmonary TB with negative sputum smear for AFB or had other indications for bronchoscopy, were included in the study. Then, fiberoptic bronchoscopy was done and BAL fluid was obtained from all patients. The study patients were divided into three groups as follows: Group 1: patients with positive sputum culture or BAL fluid culture for AFB who were considered as pulmonary TB group. Group 2: patients with negative results for TB, having lung diseases other than TB, (considered as non-tuberculous lung disease group). Group 3: those without pulmonary disease and TB which considered as the control group. Mean ADA levels in BAL fluids were measured in these groups and then compared with each other.

Results: Sixty-three patients were enrolled in the study among which 15 cases (mean age: 64.06±19.37 yrs) had pulmonary TB, 33 (mean age: 56.18±18.60 yrs) had pulmonary diseases other than TB and 15 cases (mean age: 42.13±21.45 yrs) were considered as controls. Mean ADA level in BAL fluid was 4.13±2.55 IU/L, 2.42±1.06 IU/L and 1.93±0.88 IU/L in TB group, non-tuberculous lung disease group and control group, respectively. This rate was significantly higher in the pulmonary TB group compared to the other two groups ($p=0.00$). Using Roc curve with a cut-off value of 3.5 IU/L, the highest sensitivity (57%) and specificity (84%) were obtained in diagnosis of TB.

Conclusion: The results showed that although ADA activity in BAL fluid of pulmonary TB patients was higher than those seen in other diseases, a negative test does not rule out pulmonary TB. Thus, more research is required to find more precise diagnostic methods in this regard. (Tanaffos 2008; 7(2): 45-49)

Key words: Tuberculosis, Adenosine Deaminase, Bronchoalveolar lavage, Sputum smear.

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INTRODUCTION

Tuberculosis (TB) is one of the world's most common infectious diseases and is the second cause of death after acquired immunodeficiency syndrome (AIDS) (1). Approximately 2 billion people are infected with the tubercle bacillus and 8.8 million new cases of TB occur annually (2). TB is more common in developing countries and is one of the major causes of wasting man power (3).

Despite the presence of standard diagnostic methods, diagnosis of TB is still problematic. Although the diagnostic keys of pulmonary TB are clinical symptoms and chest-x-ray findings, diagnosis is based on finding acid-fast bacilli (AFB) in sputum smears and definite diagnosis is based on the isolation and culture of *Mycobacterium tuberculosis* in sputum samples. Since the sensitivity of sputum smear is 40-70% and a 4-8 week period is required to prepare the sputum culture results, delay is unacceptable in emergency situations. Thus, it is necessary to find faster methods with higher sensitivity (4).

However, polymerase chain reaction (PCR) is a rapid diagnostic method for pulmonary TB but it is not cost-effective and few centers use it (5).

Adenosine deaminase (ADA) is an enzyme involved in purine catabolism, catalyzing the pathway from adenosine to inosine and is known as a cellular immunity marker. Physiologic activity of this enzyme is due to its release from differentiating lymphocytes (6). ADA activity has been assessed in various fluids of TB patients for diagnosis of pleural, pericardial and peritoneal TB (7-10). However, few studies have been done to assess ADA activity in bronchoalveolar lavage (BAL) fluid and its diagnostic value is still unknown. Therefore, we decided to measure ADA activity in BAL fluid of smear-negative pulmonary TB patients to assess its diagnostic value.

MATERIALS AND METHODS

A descriptive study was performed at the Shahid Sadoughi Hospital in Yazd, from 2005 to 2006. Sixty-three patients suspected of having pulmonary TB with negative sputum smears for AFB or had other indications for bronchoscopy were included in the study. After approving the proposal by the research committee, the forms for recording demographic data were completed. Then, sputum samples of all patients were sent to the laboratory for TB culture. A written consent was obtained from each patient and after 4h-fasting and local administration of lidocaine 2% spray, a fiberoptic bronchoscopy (Olympus bronchoscope type 1T20, Japan) was done. BAL fluid was obtained from a pulmonary lobe with the most involvement seen on chest x-ray and a right middle pulmonary lobe in patients with a diffuse involvement or a normal chest-x-ray. To obtain BAL fluid, 150 cc normal saline was injected and the returned fluid was collected via a suction and sent to the laboratory for AFB-smear and culture. To assess ADA activity, the samples were centrifuged and kept at -21°C. Then ADA levels in BAL fluids were compared to each other by ADA kit (Shim Enzyme CO.). ADA activity was measured by Giusti's colorimetric method. The first step is deamination of adenosine and releasing ammonia. The second reaction is catalyzed by glutamate dehydrogenase accompanying an allosteric activator. Low light absorption at 340 nm has a direct relationship to ADA activity. By this method, ADA activity can be measured up to 100 IU/L. Those patients who had positive sputum cultures or BAL cultures for AFB, were selected as the pulmonary TB group; those who had other forms of pulmonary diseases and were negative for TB were included in the non-TB lung disease group. Individuals, in which TB and other pulmonary diseases were ruled-out, were selected as the control group.

Questionnaires were filled for all three groups and

mean ADA levels in BAL fluids were measured and compared. Data were analyzed by independent t-test, ANOVA and Fisher's exact test using SPSS software ver.11.5.

RESULTS

Sixty-three patients were enrolled in this study. Fifteen patients (5 males, 10 females; mean age: 64.06 ± 19.37 yrs) had pulmonary TB, 33 (22 males, 11 females; mean age: 56.18 ± 18.60 yrs) had non-TB lung disease and 15 cases (10 males, 5 females; mean age: 42.13 ± 21.45 yrs) were controls. Among non-TB lung disease patients, 5 patients had interstitial lung disease (1 Wegener's disease, 2 cases of usual interstitial pneumonia, 2 cases of sarcoidosis and 1 silicosis), 14 had lung cancer (6 adenocarcinomas, 4 squamous cell carcinomas, 1 small cell carcinoma, 2 non-Hodgkin's lymphomas and 1 primary pulmonary Hodgkin's lymphoma), 4 had pneumonia and 10 patients had chronic obstructive pulmonary disease (COPD).

Sputum culture, BAL smear and BAL culture were positive in 5, 8 and 11 patients, respectively, in pulmonary TB group. As a whole, all TB patients had positive sputum culture or positive-BAL culture (Table 1).

Table 1. Frequency of patients with pulmonary TB based on sputum smear sputum culture and BAL culture.

	Smear		Culture	
	Negative No (%)	Positive No (%)	Negative No(%)	Positive No(%)
Sputum	15 (100)	0 (0)	10 (67)	5 (33)
Bronchoalveolar lavage fluid	7 (47)	8 (53)	4 (27)	11 (73)

In the two other groups, sputum smear and culture and BAL culture were negative for TB.

ADA levels in BAL fluids of pulmonary TB group, non-TB lung disease group, and control group

were 4.13 ± 2.55 IU/L, 2.42 ± 1.06 IU/L and 1.93 ± 0.88 IU/L respectively.

One-way ANOVA showed a significant difference in mean ADA level among the 3 groups ($p=0.000$). Tukey test also showed a significant difference between TB patients and non-TB lung disease group ($P=0.02$) and also TB patients and the control group ($P=0.01$) but no significant difference was detected between non-TB lung disease patients and the control group ($p=0.557$) (Table 2).

Table 2. Mean and SD of ADA level in BAL of patients in 3 study groups

Group	Mean ADA(IU/L)	SD	Maximum	Minimum
Pulmonary TB	4.13	2.55	10	1
Non-TB lung disease	2.42	1.06	5	1
Control	1.93	0.88	4	1

Based on the results, ADA level was 2.43 ± 1.23 IU/L in women and 3.11 ± 2.17 IU/L in men; independent t-test did not show a significant difference between the two groups ($p=0.11$, $t=1.58$).

To determine the best predictive value of ADA levels in diagnosis of pulmonary TB, a ROC curve was used which did not show a significant difference ($p=0.1016$). At the designated cut-off level of 3.5 IU/L, the highest sensitivity and specificity for diagnosis of TB was found (specificity=84%, sensitivity=57%). Using cut-off point in ADA=2.2 IU/L (mean ADA+2×SD) in the control group, the sensitivity, specificity, positive-predictive and negative-predictive values were 73%, 67%, 41% and 89%, respectively.

DISCUSSION

The results showed a significant difference in ADA levels among three groups and it was significantly higher in TB patients than the other two

groups. In a study by Kayacan et al., ADA level in BAL fluids of pulmonary TB patients, non-TB lung disease patients (like interstitial lung disease, lung cancer, pneumonia and COPD) and controls was 3.1 ± 2 IU/L, 0.4 ± 0.5 IU/L and 0.2 ± 0.4 IU/L, respectively. ($p < 0.001$) (4).

Orphanidou et al. compared ADA activity and lysozyme levels in BAL fluid of smear-negative pulmonary TB patients and non-TB lung disease patients and found that there was no significant difference in lysozyme level of BAL fluids between the two groups but ADA level in BAL fluids of pulmonary TB patients was significantly higher than that of non-TB lung disease patients ($p < 0.001$) (11). In a study conducted by Kubota et al., mean ADA level in BAL fluid of miliary TB patients, sarcoidosis patients, idiopathic interstitial pneumonia patients and control group was 5.02 ± 3.75 IU/L, 1.06 ± 0.99 IU/L, 0.21 ± 0.43 IU/L and 0.3 ± 0.51 IU/L, respectively, and ADA level in BAL fluid of miliary TB patients was higher than that of other groups ($p < 0.01$) (12). However, Reechaipichitkul et al. compared ADA levels in BAL fluid of pulmonary TB patients, lung cancer patients and those with other forms of pulmonary diseases and found no significant difference among these three groups ($p = 0.56$) (13). ADA is an enzyme found in peripheral blood and tissue lymphocytes and is increased in BAL fluid in diseases with lymphocytic reactions like sarcoidosis and hypersensitivity pneumonia (14,15). Inflammation due to TB in lung parenchyma is a type of lymphocytic inflammation and is expected to increase in pulmonary TB patients. With cut-off value of 3.5 IU/L, sensitivity=57% was obtained which was a low rate. In a study by Kayacan et al., who used cut-off point of the control group, the ADA level of BAL fluid for diagnosis of pulmonary TB in negative-sputum smear patients showed sensitivity=100% and specificity=85%, positive predictive value=76% and negative

predictive value=100% (4). Other studies showed controversial results (11-13). Furthermore, ADA activity assessed in pleural and peritoneal fluids and CSF had different diagnostic values (7-10). For example, studies conducted in regions with high incidence of TB found measurement of ADA activity to be a valuable method for diagnosis of pleural TB (10, 16); in contrast, in low TB incidence regions it is not a precise method for TB diagnosis (17). This is also true about ADA level in BAL fluid. It seems that this laboratory assessment is remarkably dependent on the method of measurement, materials and location. For instance, in some studies Guisti's method has been used to measure ADA (4). The difference between our results and other studies can be somehow related to different methods of measurement.

At the designated cut-off level=3.5 IU/L, a specificity=84% was obtained which indicated that ADA>3.5 IU/L can confidently rule out non-TB pulmonary diseases.

CONCLUSION

Although ADA level in BAL fluid of patients with pulmonary TB is significantly higher than that of other pulmonary diseases and is a rapid and cost-effective method, its sensitivity is not able to rule out pulmonary TB. Therefore, studies with a larger sample size are recommended.

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REFERENCES

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country.

- WHO Global Surveillance and Monitoring Project. *JAMA* 1999; 282 (7): 677- 86.
2. Kamholz SL. Drug resistant tuberculosis. *J Assoc Acad Minor Phys* 2002; 13 (2): 53- 6.
 3. Sudre P, ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. *Bull World Health Organ* 1992; 70 (2): 149- 59.
 4. 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 (7): 536- 41.
 5. Richeldi L, Barnini S, Saltini C. Molecular diagnosis of tuberculosis. *Eur Respir J Suppl* 1995; 20: 689s- 700s.
 6. Ungerer JP, Oosthuizen HM, Bissbort SH, Vermaak WJ. Serum adenosine deaminase: isoenzymes and diagnostic application. *Clin Chem* 1992; 38 (7): 1322- 6.
 7. Dwivedi M, Misra SP, Misra V, Kumar R. Value of adenosine deaminase estimation in the diagnosis of tuberculous ascites. *Am J Gastroenterol* 1990; 85 (9): 1123- 5.
 8. Fernandez-Rodriguez CM, Perez-Arguelles BS, Ledo L, Garcia-Vila LM, Pereira S, Rodriguez-Martinez D. Ascites adenosine deaminase activity is decreased in tuberculous ascites with low protein content. *Am J Gastroenterol* 1991; 86 (10): 1500- 3.
 9. Hillebrand DJ, Runyon BA, Yasmineh WG, Rynders GP. Ascitic fluid adenosine deaminase insensitivity in detecting tuberculous peritonitis in the United States. *Hepatology* 1996; 24 (6): 1408- 12.
 10. Orphanidou D, Gaga M, Rasidakis A, Dimakou K, Toumbis M, Latsi P, et al. Tumour necrosis factor, interleukin-1 and adenosine deaminase in tuberculous pleural effusion. *Respir Med* 1996; 90 (2): 95- 8.
 11. Orphanidou D, Stratakos G, Rasidakis A, Toumbis M, Samara J, Bakakos P, et al. Adenosine deaminase activity and lysozyme levels in bronchoalveolar lavage fluid in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1998; 2 (2): 147- 52.
 12. Kubota M, Katagiri M, Yanase N, Soma K, Tomita T. Measurement of adenosine deaminase activity in bronchoalveolar lavage fluids as a tool for diagnosing miliary tuberculosis. *Nihon Kyobu Shikkan Gakkai Zasshi* 1996; 34 (2): 139- 44.
 13. Reechaipichitkul W, Lulitanond V, Patjanasontorn B, Boonsawat W, Phunmanee A. Diagnostic yield of adenosine deaminase in bronchoalveolar lavage. *Southeast Asian J Trop Med Public Health* 2004; 35 (3): 730- 4.
 14. Kubota M, Katagiri M, Imasaki T, Yanase N, Soma K, Tomita T. Adenosine deaminase activity in bronchoalveolar lavage fluid of sarcoidosis patients. *Nihon Kokyuki Gakkai Zasshi* 1999; 37 (5): 374-9.
 15. Albera C, Mabritto I, Ghio P, Solidoro P, Marchetti L, Pozzi E. Adenosine deaminase activity and fibronectin levels in bronchoalveolar lavage fluid in sarcoidosis and tuberculosis. *Sarcoidosis* 1993; 10 (1): 18- 25.
 16. Bañales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, Salazar-Lezama M. Adenosine deaminase in the diagnosis of tuberculous pleural effusions. A report of 218 patients and review of the literature. *Chest* 1991; 99 (2): 355- 7.
 17. van Keimpema AR, Slaats EH, Wagenaar JP. Adenosine deaminase activity, not diagnostic for tuberculous pleurisy. *Eur J Respir Dis* 1987; 71 (1): 15- 8.