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Serum adenosine deaminase activity and the total antioxidant capacity of plasma in pulmonary tuberculosis and non-tuberculosis pulmonary disease

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Aim: The aim of this study was to investigate the level of serum adenosine deaminase (ADA) and the total antioxidant capacity (TAC) in pulmonary tuberculosis (PTB), non-tuberculosis pulmonary disease (non-PTB) and healthy subjects.

Materials and methods: Serum ADA activity was measured using the Giusti and Galanti method, and the total antioxidant capacity of plasma was determined by the ferric reducing ability of plasma (FRAP) test.

Results: The serum ADA levels were significantly higher ($P < 0.001$) in pulmonary TB (PTB, 19.78 ± 7.09 U/L), as well as in non-PTB patients (14.78 ± 4.65 U/L) when compared to healthy controls (10.02 ± 1.99 U/L). The sensitivity and specificity were found to be 71.7% and 63.3%, respectively, in distinguishing PTB from non-PTB. In distinguishing PTB from healthy subjects, the sensitivity and specificity were 87% and 93.3%, respectively. The TAC was significantly lower in PTB (485.2 ± 190.0 μM) and non-PTB patients (588.3 ± 195.8 μM), when compared to the controls (784.3 ± 190.0 μM; $P < 0.001$). Plasma antioxidative activity decreased in PTB and non-PTB patients when compared to the controls.

Conclusion: We concluded that serum ADA activity is not a useful test to differentiate pulmonary TB from other respiratory diseases. The TAC is low in pulmonary TB, therefore supplementation with a suitable anti-oxidant may be useful.

Key words: Tuberculosis, adenosine deaminase activity, ADA, total antioxidant capacity

Introduction

Annually, more than 8 million people develop tuberculosis (TB), and approximately 1.8 million cases result in death (1). TB has a long incubation period, with the timeline for transition from infection to expression lastingmonths or decades. Therefore, it is not surprising that identification of interspersion transmission events by classical epidemiological tools, like contact tracing, suffer from recall and observer biases (2). The introduction of chemotherapeutic and prophylactic measures had led to a substantial reduction in deaths, which was maintained for decades. Regardless, TB is still responsible for the most human deaths caused by a single infectious agent (3). Pulmonary TB continues to be a major health problem in the Sistan and Balouchistan provinces of Iran, where it is the most prevalent disease.

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Adenosine deaminase (ADA) (E.C. 3.5.4.4.), an enzyme responsible for the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, is involved in the proliferation and differentiation of lymphocytes, particularly, the T subtype (4). It also plays a role in the differentiation of lymphoid cells and the maturation of monocytes to macrophages. The presence of ADA in pericardial and other body fluids reflects the activity of the cellular immune response in the respective compartments, and in particular, the activation of T lymphocytes and macrophages. ADA has also been considered a marker of cell-mediated immunity (5). Increased serum ADA activities were observed in many infectious diseases caused by microorganisms, which mainly infected macrophages. Since Mycobacterium tuberculosis infects lung macrophages, ADA may be released and detected in the serum of patients with TB. Mycobacteria can induce reactive oxygen species (ROS) production by activating phagocytes (6), and though these are important elements of the host's defense against mycobacteria, an enhanced ROS generation may promote tissue injury and inflammation. This may further contribute to immunosuppression, particularly, in those with impaired antioxidant capacity, such as HIV infected patients (7). Furthermore, malnutrition, which is commonly present in patients with TB, may contribute to their impaired antioxidant capacity.

Direct (unconcentrated) sputum smears microscopy is the primary test for diagnosing pulmonary tuberculosis in developing countries. This method is quick and inexpensive, but has relatively low sensitivity. The in-vitro culture of mycobacterium tuberculosis bacilli is the golden standard, but is time-consuming (8). The polymerase chain reaction (PCR) assay has shown good sensitivity and specificity in several studies (9, 10), but it requires extreme precision, accuracy, sophisticated lab equipment, and skilled technicians. A rapid diagnostic test may be helpful for a diagnosis of pulmonary disease (11). Therefore, in this study we measured the level of serum ADA to determine if it was a distinguishing factor between pulmonary TB (PTB) and non-PTB pulmonary disease (non-PTB) patients.

Patients and methods

Patients

This case-control study was performed from February 2006 to May 2007 at the Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital, Zahedan University of Medical Sciences, Zahedan, Iran. The project was approved by the Ethical Committee of Zahedan University of Medical Sciences, and an informed consent was given by all patients and healthy individuals.

Blood samples were obtained from PTB (n = 85; 37 male, 48 female), non-PTB (n = 220; 120 male, 100 female), and healthy controls (n = 83; 50 male, 33 female). The diagnosis of PTB was based on clinical, radiological, sputum Acid Fast Bacillus (AFB) smear positivity, as well as culture and response to anti-tuberculosis chemotherapy, as described previously (11, 12). Non-PTB cases were identified as patients showing signs and symptoms of acute pneumonia (confirmed by chest X-ray), and for whom 3 consecutive sputum smears were negative for AFB. The diagnosis of these patients was based on a negative culture for mycobacterium tuberculosis and/or clinical improvement and radiological resolution on follow-up (11, 12). Normal subjects were healthy individuals showing no signs, symptoms, or history of pulmonary infections. The mean ages of PTB patients, non-PTB patients, and healthy individuals were 57.9 ± 18.5, 56.8 ± 17.1, and 38.4 ± 8.7 years, respectively. Age was significantly different between PTB and healthy individuals ($P < 0.0001$), while there was no significant difference between the other patient groups ($P = 0.83$). The Body Mass Index (BMI) was not significantly different between PTB (20.2 ± 4.4 kg/m^2) and non-PTB (20.9 ± 4.4 kg/m^2) ($P = 0.339$) patients, whereas it was significantly lower in PTB and non-PTB when compared to normal subjects (25.4 ± 3.8 kg/m^2) ($P < 0.05$).

Measurement of Serum ADA activity and TAC

For the determination of ADA activity, serum samples were obtained. For TAC measurement, blood was drawn into anti-coagulated tubes containing sodium EDTA, and plasma was separated by centrifugation at 1500 × g for 10 min at +4 °C, and stored at -20 °C until its use. ADA activity was measured using the Giusti and Galanti method based

on the Bertholet reaction (13). Briefly, the indophenol complexes formed when ammonia was released from adenosine and was quantified using a spectrophotometer at a wavelength of 620 nm. One unit of ADA was defined as the amount of enzyme required to release one micromole of ammonia per minute from adenosine at standard assay conditions. ADA activity was expressed as units per liter (U/L) in the serum.

Plasma total antioxidant capacity (TAC) was determined by the ferric reducing ability of plasma (FRAP), which measures the ability of plasma to reduce Fe^{3+} to Fe^{2+} (14).

Statistical analysis

The results were expressed as mean ± S.D. Commercial software (SPSS for Windows, v. 17) was used for statistical analysis of variables. The normality of the data was checked using Kolmogorov-Smirnov test. Data was analyzed using one-way ANOVA, and the Tukey multiple comparison test. The P-values < 0.05 were considered statistically significant. Pearson's correlation coefficient was used to determine the relationship between variables. Receiver operating characteristic (ROC) curves, and the area under the ROC curves (AUC) with 95% confidence intervals, were calculated for evaluating the optimum cut-off level. The optimum cut-off level was determined by selecting points of test values that provided the greatest sum of sensitivity and specificity. Sensitivity, specificity, positive predictive value, and negative predictive value were determined.

Results

Serum ADA activity was significantly higher (Figure 1, P < 0.001) in PTB (19.78 ± 7.09 U/L) as well as non-PTB (14.78 ± 4.65 U/L) when compared to healthy controls (10.02 ± 1.99 U/L). Additionally, serum ADA activity was significantly higher in PTB when compared to non-PTB controls (P < 0.05).

The optimum cut-off value for ADA was 16.5 U/L in distinguishing PTB from non-PTB (Figure 2A). The sensitivity, specificity, positive predictive, and negative predictive value were found to be 71.7%, 63.3%, 42.8%, and 84.4%, respectively (Table 1).

The optimum cut-off value to differentiate ADA activity between PTB and healthy controls was 12.5 U/L (Figure 2B). The sensitivity, specificity, positive predictive value, and negative predictive values were 87%, 93.3%, 92.5%, and 84.1%, respectively (Table 1).

Plasma TAC of PTB (485.2 ± 190.0 μM) and non-PTB subjects (588.3 ± 195.8 μM) were significantly lower than that of healthy subjects (784.3 ± 190.0 μM) (Figure 3, P < 0.001).

There was no correlation between ADA activity and TAC in pulmonary TB (r = -0.0256, P = 0.814), and non-TB pulmonary disease (r = -0.0777, P = 0.251).

Discussion

Adenosine deaminase (ADA) is essential for the differentiation of lymphoid cells; in particular, T cells, and is found to play an important role in the maturation of monocytes to macrophages (15). Also, ADA is considered to be an indicator of cell-mediated immunity (16). Monocyte/macrophage activation by intracellular infection and inflammatory diseases leads to the release of ADA and elevated levels in serum. Increased serum ADA levels in pulmonary TB may result from a stimulation of cell-mediated immunity (17).

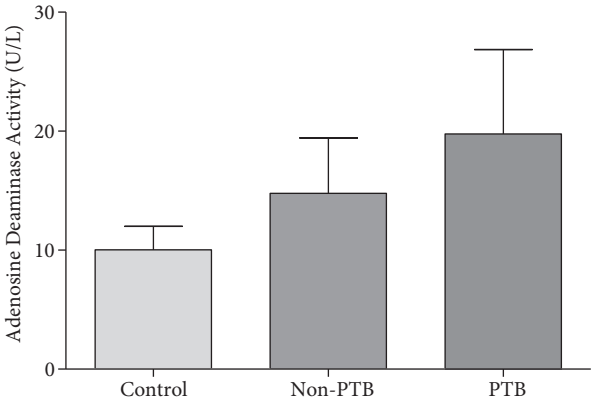

Figure 1. Serum ADA activity in pulmonary tuberculosis (PTB; 19.78 ± 7.09 U/L), non-tuberculosis respiratory disease (non-PTB; 14.78 ± 4.65 U/L) and healthy controls (10.02 ± 1.99 U/L). Serum ADA activity was significantly higher in pulmonary tuberculosis, as well as non-tuberculosis respiratory disease, when compared to healthy controls (P < 0.001).

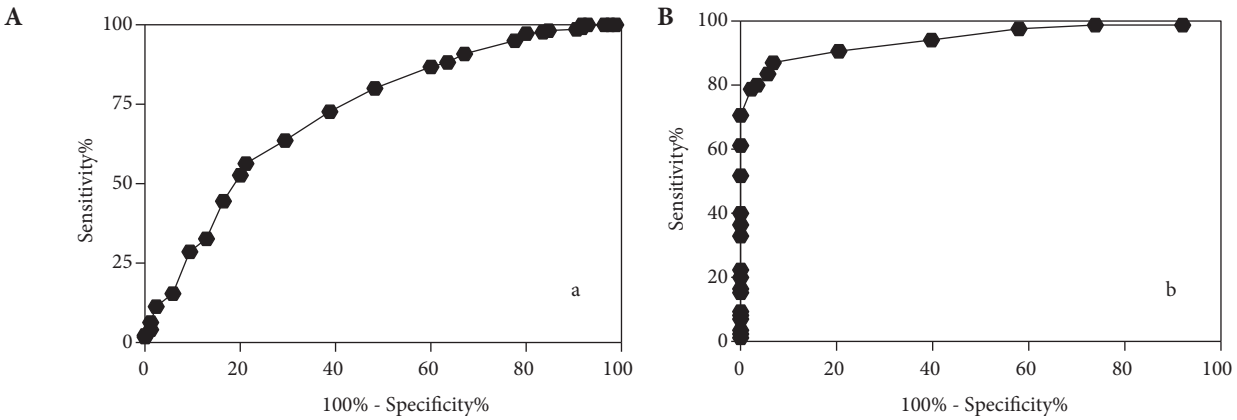

Figure 2. The ROC curve analysis for determining the optimum cut-off point in distinguishing PTB from non-PTB patients (**2A**) and the controls (non-PTB patients and healthy subjects) (**2B**). The optimum cut-off points were found to be 12.5 U/L and 16.5 U/L, respectively, in distinguishing TB from non-PTB, and TB from control subjects, respectively.

Table 1. The optimum cut-off level of ADA was found to be 16.5 U/L in distinguishing pulmonary tuberculosis (PTB) from non-TB pulmonary disease (non-PTB). Sensitivity, specificity, positive predictive value and negative predictive value were found to be71.7%, 63.3%, 42.8% and 84.4%, respectively.

Total	non-PTB	PTB	Disease ADA (U/L)
140	80	60	≥ 16.5
165	140	25	<16.5
305	220	85	Total

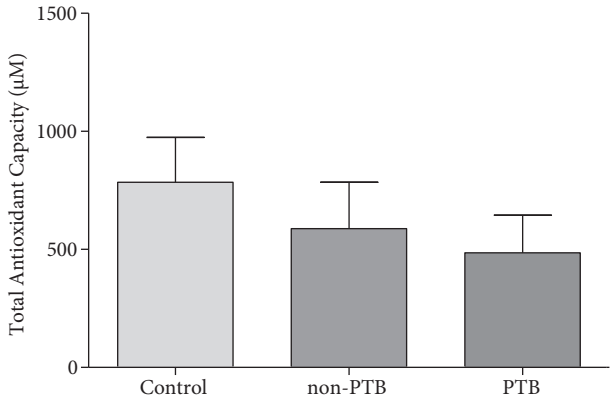

Figure 3. Comparison of the TAC of plasma in pulmonary tuberculosis (PTB), non-tuberculosis respiratory disease (non-PTB) and healthy subjects. The TAC was significantly lower in pulmonary tuberculosis, as well as non-tuberculosis respiratory disease when compared to healthy controls (P < 0.001).

We report that the serum concentration of ADA was significantly higher (P < 0.001) in pulmonary TB (19.78 ± 7.09 U/L), as well as non-TB pulmonary disease (14.78 ± 4.65 U/L) when compared to the control group (10.02 ± 1.99 U/L). A number of groups have reported different values for normal human serum ADA activity: 5.9 ± 17.6 U/L (18), 13.04 ± 3.3 (19), 8.58 ± 4.38 U/L (20), 14.0 ± 0.5 U/L, 10.31 ± 0.58 U/L (21), 16.5 ± 3.18 U/L(22), 11.1 ± 3.0 U/L (23), and 2.23 ± 1.0 U/L (24). Our findings were similar to those of previous studies (25-27).

For several years, the ability to differentiate the diagnosis of PTB and non-PTB was an important issue for clinicians. As such, many techniques have been used to address this aim. Serum ADA activity is one such method, for which there have been varying results. Lamsal et al. found a cut-off value of 25 U/L, a test sensitivity of 72.41%, and a specificity of 81.53% (25). Kuyucu et al. reported a serum ADA level of greater/equal to 53.76 U/L, a sensitivity of 100%, and a specificity of 90.7%, while indicating a positive predictive value of 58.8%, and a negative predictive value of 100% in children with TB (17). Bhargave et al. and Al-Shammary et al. reported the cut-off value of serum ADA levels in tuberculosis patients as 78.12 IU/L and 32.8 U/L, respectively (5, 27). ADA activity has been shown to be higher in children with active

TB, than in children with bacterial or viral pneumonia (28). In our study, the optimum cut-off point of ADA for distinguishing PTB and non-PTB subjects was found to be 16.5 U/L using the ROC curve. Therefore, according to our divisive results, it can be concluded that serum ADA activity might not be a suitable test for distinguishing pulmonary TB and non-TB pulmonary disease.

Free radical formation is a consequence of a variety of essential biochemical reactions and could be unregulated under pathophysiological conditions (29). Antioxidants play an important physiological role counteracting free radicals and preventing cellular damage. Lung inflammation occurs during TB infection and is a source of free radical generation. It was reported that patients with advanced TB had increased levels of lipid peroxidation products and malondialdehyde (30). Our study also showed that plasma TAC was significantly decreased in patients with PTB or non-PTB when compared to the controls. This finding was previously reported by Wiid et al., who found that the antioxidant status was decreased in active TB patients when compared to controls, but was subsequently increased during the therapeutic procedure. This suggests that the disease contributed to the depleted antioxidant status of the patients, not only via the inflammatory side effects of the disease, but perhaps, also through the lowered nutritional intake as a consequence of the disease. In TB, oxidative stress is a result of tissue inflammation and free radical rupture from activated macrophages (31). These free radicals may cause pulmonary inflammation if they are not scavenged by antioxidants. Therefore, TAC is a suitable indicator of the free radical load, and antioxidant supplement might be a good therapeutic protocol in TB and non-PTB patients with a low TAC.

In conclusion, although serum the ADA measurement is simple and inexpensive, it is not a useful test to differentiate PTB from other respiratory diseases, due to its low sensitivity and specificity. However, TAC may be a valid approach to determine the antioxidant status of TB and non-PTB patients.

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