Adenosine Deaminase Activity in sera and lymphocytes of patients with Brucellosis

Mahmoood M Atia* Ph.D

Tahseen A Zydan** Ph.D

Ali M S Al-Doori *** PhD

Wissam M Atia* BSc

Abstract:

Background: Adenosine deaminase enzyme has been used as marker for infectious diseases e.g. tuberculosis and AIDS. Its activity is found to be elevated in diseases in which there is a cell mediated immunity. This work was carried out to study adenosine deaminase activity in patients with brucellosis.

Methods: Blood samples were collected from patients with brucellosis 35 samples before treatment and 30 after four days starting treatment) and 25 samples from apparently "healthy" controls. Adenosine deaminase was determined. Analysis of variance was used to examine the association of adenosine deaminase with infection.

Results: Significant difference was noticed in adenosine deaminase enzyme between patients with infection and controls, and, also, patients after treatment. No such significant difference was observed in adenosine deaminase between patients after treatment and control.

Conclusion: Adenosine deaminase activity was higher in patients with brucellosis. Further studies are needed to explain the causes and mechanisms of increased level of adenosine deaminase.

Key word: Adenosine deaminase enzyme, Brucellosis, Iraq

Introduction:

Adenosine has been established as an important inhibitor of immune system. Its tissue levels can be modulated by adenosine deaminase (ADA) [1, 2]. ADA is expressed in two iso-enzymes: ADA1 synthesized in all tissues and is found free or associated with CD26 [3, 4]. ADA2 exclusively synthesized in monocytes and macrophages, has been used as a marker of infectious diseases such as tuberculosis [5, 6], AIDS [7] and typhoid fever [3]. ADA activity is found to be elevated in those diseases in which there is cell mediated immunity [8, 9].

This work was carried out to study ADA activity in patients with Brucellosis.

Materials & methods:

Blood samples were collected from 35 patients before treatment (group 1), 30 patients after four days starting treatment (group 11) and 25 blood samples were collected from "apparently" healthy subjects as control group. Brucellosis was diagnosed by Rose–bengal and 2-mercaptoethanol tests [10].

Lymphocytes were separated by ficoll gradient centrifugation technique (Sigma Chemical Co.). The red blood cells were removed by hypotonic lyses. The separated lymphocytes were washed three times with Hanke balanced salt solution (HBSS), the suspension of the cells in HBSS was centrifuged at 800x gm for 10 minutes, and supernatant were discarded. The sediment was suspended in one ml distilled water and suspension of cells was frozen and thawed six times. The centrifuged supernatant was then analyzed for enzyme activity and protein determination. Protein estimation was performed using the method of Lowery et al [11] and the result was expressed in mg/L. Serum and lymphocytes ADA activity were determined at 37 ^{O} C according to the method of Giusti and Galanti [12]. Results were expressed as international unit of enzyme activity of serum and of specific enzyme activity / mg protein of lymphocytes.

Analysis of variance was used to examine the association of dependent variable (ADA) with infection [13]. P value less than 0.05 was considered significant.

Results:

Table 1 shows the distribution of ADA activity (serum and lymphocytes) in the studied groups. A significant statistical differenced was noticed in ADA activity (serum and lymphocyte) between group 1 and controls ($p < 0.05$), while no such difference was observed in ADA activity between group 11 and controls ($p > 0.05$). A significant statistical difference was demonstrated in ADA activity between group 1 and group 11 ($p < 0.05$).

Table 1 Distribution of ADA in the studied groups

		ADA activity		
Group	**No. examined**	**Serum**		**Lymphocytes**
		IU/L		**IU/gm protein**
Group I	35	49.16 ± 20		12.93 ± 5.6
Group II	30	15.55 ± 5.8		7.6 ± 4.6
Controls	25	13.4 ± 1.09		7.37 ± 4.1

Group I Vs. controls $p < 0.05$

Group II Vs. controls $p > 0.05$

Group 1 Vs. Group II $p < 0.05$

Discussion:

The role of ADA in cellular immune function was highlighted following the discovery of reduced levels in patients with sever combined immunodeficiency [13]. ADA is found only in monocytes and macrophages [14]. The predominant isoenzyme in the sera is ADA in all infectious diseases [15]. It has strongly suggested that serum ADA activity reflects monocytes / macrophages activity or turn over in different diseases [16, 17].

This study revealed that ADA activity (serum and lymphocytes) was significantly higher in patients with brucellosis (before treatment) than controls, while it was not in patients after treatment. The increased serum ADA activity in patients with brucellosis may reflect the phagocytic activity of macrophages. On the other hand, the increased lymphocytes ADA activity may, also, reflecting the differentiation of lymphoblastoid cells. Several reports demonstrated high ADA activity in many infectious diseases caused by microorganisms infecting mainly macrophages e.g. tuberculosis [5, 6], cutaneous leishmaniasis [18] and visceral leishmaniasis [5]. Brucella is an intracellular pathogen [19].

According to our knowledge this is the first report on ADA activity in Brucellosis in Iraq. To explain the cause and mechanisms of increased levels of ADA activities in Brucellosis, further studies are needed.

References:

1-Cronstein BN, Kramer SB, Weissmann G, Hirschhorn R. Adenosine: A physiological modulator of superoxide anion generation by human neutrophils. J Exp Med 1983; 158:1160-1177.

2-Cronstein BN, Levine RI, Belanoff J, Weissmann G. Hirschhorn R. Adenosine: an endogenous inhibitor of neutrophil – mediated injury to endothelial cells. *J Clin Invest 1986; 78:760-770.*

3-Dong RP, Kameoka J, Hegen M, Tanaka T, Xn Y, Scholssmsn SF, Morimoto C. Characterization of adenosine deaminase binding to human CD26 on T cells and its biological role in immune response. *J Immunol 1997; 156: 1349-1355.*

4-Ungerer JP, Burger HN, Bissbort SH, Vermaak WJH. Adenosine deaminase isoenzyme in typhoid fever. *Eur J Clin Microbiol Inf Dis 1996; 15:510-512.*

5-Gakis C, Calia GM, Natana AGU, Ortu AR, Contu A. Serum and pleural adenosine deaminase activity. Chest 1991; 99: 1555-1556.

6-Valdez L, Alvare, SanJose 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; 38: 1322-1326.

7-Gakis C, Calia GM, Natana AGV, Prinio D, Serra G. Serum adenosine deaminase activity in HIV positive subjects. A hypothesis on significance of ADA2. Panminerua Medica 1989; 3: 107-113.

8-Galanti B, Nardiello S, Russ M, Fiorentino F. Increased lymphocyte adenosine deaminase in typhoid fever. *Scand J Infect Dis 1981; 13:47-50.*

9-Piras MA, Gakis C, Budroni M, and Andreoni G. Immunological studies in Mediterranean spotted fever. Lancet 1982; 1 (8283): 1249.

10-Diaz R, Maravi-Poma E, Rivera A. Comparison of counter immunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. Bul WHO 1976; 53: 417-424.

11-Lowery OH, Rosebroygw NL, Farr AL,Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem 1951; 193: 265-275.*

12-Giusti G, Galanti B. Adenosine deaminase, colometeric method. In: Bergmeyer (editor). Method of enzyme analysis. Vol.4, 3rd edition. Verlac Chemie, Weinheim 1984. pp. 315-323.

13-Daniel WW. Biostatistics: A foundation for analysis in health sciences. 7th edition. New York. John Wiley and Sons Inc., 1999.

14-Vander WM, Kelly WN.Adenosine deaminase deficiency and severe combined immunodeficiency. Life Sciences 1977; 20: 1645-1650.

15-Zuckerman SH, Olson JM, Douglas SD. Adenosine deaminase activity during in vitro culture of human peripheral blood monocytes and pulmonary alveolar macrophages. Exp Cell Res 1980; 129: 281-287.

16-Ungerer JP, Oosthuizen HM, Bisbort SH, Vermaak WJ. Seum adenosine deaminase isoenzyme and diagnostic application. Clin Chem 1992; 38: 1322-1326.

17-Stancikona M, Rovensky J. Effect of cyclosprin on activity of cytidine deaminase and adenosine deaminase in serum and polymorphonuclear leukocytes of patients with rheumatoid arthritis. Int *J Tiss Reac 1993; 15:169-174.*

16-Erel O, Kocyigit A, Gurel MS, Bnhut U, Seyrek A, Ozdemir Y. Adenosine deaminase activity in sera, lymphocytes and granulocytes in patients with cutaneous leishmaniasis. Mem Inst Oswaldo Rio de Janeiro 1998; 93: 490-494.

17-Pizarro-Cerda J, Moreno E, Sanguedoke V, Mega JL, Gorvel JP. Virulent Brucella abortus prevent lysosome fusion and distributed within antophagosome like compartment. Int Immun 1998; 66: 2387-2392.

---

*From Biochemistry Dept, Coll. of Med, Al-Anbar Univ

** from Chemist Dept, Coll of Science, Al-Anbar University

***From Pathology Dept, Coll of Med, Al-Anbar Univ