

Serum Adenosine Deaminase Level in Iraqi Women with Toxoplasmosis with a History of Abortion

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Abstract: Sixty women who had abortion were selected for this study. Serum was collected from the women. ELISA test was used to detect anti-*Toxoplasma* specific antibodies. Those women were divided into three groups according to the presence or absence of specific anti-*Toxoplasma* antibodies. These groups included the women with IgG (18), the women with IgM (14) and the women with both IgG and IgM (12). The other 16 women had no antibodies against *Toxoplasma*. Twenty-four healthy-looking women had been selected as controls. Serum was collected from those women and tested for anti-*Toxoplasma* antibodies. Those that revealed any antibody titer against *Toxoplasma* were excluded from the study. The activity of ADA was determined in the serum according to the method of Giusti. The mean concentration of ADA in the women who had anti-*Toxoplasma* IgM antibody was significantly higher ($P<0.0001$) than that of healthy controls and the women who had IgG, while there was no significant differences between women who had IgG antibody and healthy controls ($P>0.05$). Since a relationship exists between ADA activity and the cell mediated immune response, this result confirms the fact that *Toxoplasma* antigen induces T-lymphocytes proliferation.

Keywords: Toxoplasmosis, Abortion, Adenosine Deaminase

Introduction

Adenosine deaminase, ADA, (adenosine aminohydrolase, EC 3.5.4.4), is the enzyme that irreversibly catalyzes the hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively. The activity of this enzyme defined as the amount of enzyme that deaminates one micromole of substrate per minute, under specified steady assay conditions. The normal values of ADA in serum range from 2-17 U/L (Akalal *et al.*, 2004). This enzyme is widely distributed in animal tissues. It showed that normal blood of humans and mammals contained activity. The highest activity was found in the cecum, intestinal mucosa and spleen, while less or no activity was found in skeletal muscles, skin and bone. This enzyme is present in the cytoplasmic fraction of the cell and a certain amount is located in the nucleus (Smillie, 1957).

Studies have suggested a critical role for ADA activity in the normal development of the immune system (Thompson and Seegmiller, 1980). The conversion of adenosine and deoxyadenosine into their respective inosine nucleosides is an initial step of a series of reactions responsible for lymphocytes proliferation and differentiation. Therefore, ADA considered an indicator of cellular immunity and fundamental for the differentiation of lymphocytes. In humans, the lack of this enzyme results in severe lymphopenia and immunodeficiency, bringing the risk of an early death for the affected individuals (Blackburn and Kellems, 2005; Bollinger *et al.*, 1996).

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, ranks third in the United States for deaths caused by food-born pathogens (Hughes *et al.*, 2000). Toxoplasmosis can be transmitted across the placenta in women who have the infection, acquired

either for the first time or recurring (especially in persons who are immunocompromised) during pregnancy and it is developing asymptomatic and when passed in pregnancy period, it can cause abortion, dead birth and births with congenital anomalies according to the strain of the parasite, virulence factors and maternal immuneresponsivness (Rorman *et al.*, 2006). *T. gondii* is an important water- or food-born pathogen that is capable of causing severe disease in infants from infected mothers and in the immunocompromised patients (Roberts and McLeod, 1999). It is generally assumed that approximately 25 to 30% of the world's human population is infected by *Toxoplasma* (Montoya and Liesenfeld, 2004). Actually, the prevalences vary widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region (Pappas *et al.*, 2009). Low seroprevalences (10 to 30%) have been observed in North America, in South East Asia, in Northern Europe and in Sahelian countries of Africa. Moderate prevalences (30 to 50%) have been found in countries of Central and Southern Europe and high prevalences have been found Latin America and in tropical African countries. In Arab countries, toxoplasmosis had been investigated such as in United Arab Emirates (Mohammed and Al-Nasiry, 1996), Saudi Arabia (Al-Qurashi *et al.*, 2001) and in Egypt (Soliman *et al.*, 2001). In Iraq, many studies were published about the seroprevalence of toxoplasmosis by using different techniques like IHA, IFAT and ELISA (Kubba *et al.*, 1986; Niazi *et al.*, 1992).

Materials and Methods

Subject Selection

Patients

Sixty women who had abortion were selected for this study. They were referred to the Central Health Laboratory in Baghdad according to the physician's reports, indicating the possibility of them having toxoplasmosis. Serum and venous heparinized blood was collected from those women in a period between January 2003 and March 2003.

Controls

Twenty-four healthy-looking women had been selected as controls to compare with our patients in the same parameters of this study. Serum and venous heparinized blood was collected from those women and tested for anti-*Toxoplasma* antibodies. Those that

revealed any antibody titer against *Toxoplasma* were excluded from the study.

Enzyme Linked Immunosorbant Assay (ELISA)

The type of ELISA in this study was direct ELISA principled as the antigen of *Toxoplasma gondii* was absorbed on the ELISA plate to detect specific IgM or IgG antibody in the patient's sera when added to the well of the plate, then anti-human conjugated antibody and substrate added to get the color reaction.

This test performed by the use of ELISA kits from Omega Diagnostics Company, Scotland to detect of IgG and IgM antibodies against *Toxoplasma gondii* in the patient's sera. The patient's sera were stored for several days at -20°C until the test performed.

All preparations and assay procedure applied according to the kit manufacturer's manual.

Note: All analysis of this study were accomplished in 2003 but the writing of the manuscript was performed in 2015 because critical conditions of the editors.

Calculation and Interpretation of Results:

The average Optical Density (OD) of the low positive control was calculated and this was the Cut-Off value of the assay. The sample OD was divided by the Cut-Off value obtained above. A ratio greater than 1:1 indicated a positive result; a ratio lower than 0.9 indicated a negative result. A ratio between 0.9 and 1.1 indicated an equivocal result. An equivocal sample must be retest with a fresh new sample. In the case that the same equivocal result was obtained, the test must be repeated with a new sample after 2-4 weeks.

Adenosine Deaminase Activity Assay

The activity was determined in the serum according to the method of Giusti (Akala *et al.*, 2004).

Reagents:

All reagents were prepared at the time of assay initiation to avoid any loss or gain of ammonia in any of the reagents, which may interfere with the results.

Phosphate Buffer (50 mM)

About 4.73 gm of hydrated sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) (Riedel-de-Haen) and 5.62 gm of hydrated disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) (Riedel-de-Haen) were dissolved in DDW, bringing the final volume to 100 ml and adjusting the pH to 6.5 with phosphoric acid (H_3PO_4) (Fluka).

Buffered Adenosine Solution (21 mM Adenosine, 50 mM Phosphate)

About 15 ml of phosphate buffer was added to 140 mg of adenosine ($C_{10}H_{23}N_5O_4$) (BDH) and warmed in a 70°C water bath for 10 min then cooled under running water. The pH was adjusted to 6.5 with phosphoric acid and the volume was brought to 25 ml with phosphate buffer.

Ammonium Sulphate Stock Solution (15 mM)

About 1.982 gm of anhydrous ammonium sulphate [NH_4]₂SO₄ (Fluka) were dissolved in DDW bringing the final volume to 1000 ml.

Ammonium Sulphate Standard Solution (75 μ M; 0.15 μ lvol, NH₃/ml)

About 0.5 ml of ammonium sulphate stock solution was diluted to 100 ml with phosphate buffer.

Phenol/Nitroprusside Solution (106 mM Phenol; 0.17 mM Sodium Nitroprusside)

About 10 gm of phenol (C_6H_5OH) (DH) and 50 gm of sodium nitroprusside ($Na_3 [Fe(CN)_5NO].2H_2O$) (BDH) were dissolved in DDW bringing the final volume to 1000 ml.

1 N Sodium Hydroxide

About 40 gm of sodium hydroxide (NaOH) (BDH) was dissolved in DDW bringing the final volume to 1000 ml.

Alkaline Hypochlorite Solution (11mM NaOCl; 125 mM NaOH)

About 125 ml of 1N sodium hydroxide solution and 16.4 ml of sodium hypochlorite (NaOCl-5% w/v) (Fluka) were mixed with DDW bringing the final volume to 1000 ml.

Table 1. Experimental design for ADA estimation in the serum of women infected with *T. gondii*

Reagents	Tubes				
	Reagent blank (ml)	Standard (ml)	Adenosine blank (ml)	Sample blank (ml)	Sample (ml)
-Phosphate buffer	1.0	-	-	1.0	-
-Buffered adenosine solution	-	-	1.0	-	1.0
-Ammonium sulphate (standard solution)	-	1.0	-	-	-
-Sample (serum)	-	-	-	0.05	0.05
-DDW	0.05	0.05	0.05	-	-
Phenol nitroprusside solution	3.0	3.0	3.0	3.0	3.0
Alkaline hypochlorite solution	3.0	3.0	3.0	3.0	3.0

Assay Procedure

An adenosine blank (for the whole series) was prepared and a corresponding number of sample blanks (one for each sample, without adenosine) were also prepared according to Table 1, the sample being the serum. The calculations were made as follows:

$$E_{\text{sample}} - E_{\text{sample blank}} = A$$

$$E_{\text{adenosine blank}} - E_{\text{reagent blank}} = B$$

$$E_{\text{standard}} - E_{\text{reagent blank}} = C$$

$$\text{Enzyme activity} = ((A - B) / C) \times 50 (\text{U/L}) ; 37^\circ\text{C}$$

The contents were mixed, tubes stoppered and incubated for 60 min in a water bath at 37°C.

Then the following solutions were added.

The last two solutions were added for coloring and the contents of the tubes mixed before pipetting into the next tube. The tubes were incubated for 30 min in a water bath at 37°C. The Absorbency (A) of the sample was read using a spectrophotometer at 628 nm against DDW.

Statistical Analysis

The data were statistically analyzed using student's T-test according to Snedecor and Cochran (Hovi *et al.*, 1979) by using Excel 2003 software.

Ethical Approval

This research underwent to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Iraqi Ministry of Health also got the approval of the research by the Committee of ethical standards in the Faculty of Medicine, Al-Nahrain University, one of the colleges affiliated to the Ministry of Higher Education and Scientific Research, Iraq.

Results

ELISA Results

Sixty samples of women's serum had been tested for specific IgG and IgM antibodies to confirm the presence of toxoplasmosis in those women by using ELISA kit (Omega diagnostics).

The results showed that 44 of 60 women (73.3%) (Table 2) have antibodies against *Toxoplasma*, 18 (56.2%) (Table 3) of which have IgG antibody, 14 (43.7%) (Table 4) have IgM and 12 (27.2%) (Table 5) have both IgG and IgM. The other 16 of 60 women, although they had abortion (single or repeated), they were negative for toxoplasmosis using ELISA.

On the other hand, samples from 24 healthy looking women were collected as controls and tested for IgG and IgM specific antibodies for *Toxoplasma* by using ELISA kit. The results indicated that 20 women were negative to IgG and IgM of *Toxoplasma*, while the remaining 4 women had *Toxoplasma* antibodies.

The sensitivity and specificity of the ELISA kit had been calculated and present as 91.6% sensitivity and 55.5% specificity.

Table 2. Frequency distribution of *Toxoplasma gondii* antibody using ELISA in seropositive women with history of abortion compared with healthy controls

Type of cases	ELISA positive (%)	ELISA negative (%)	Total (%)
Abortion	44 (73.3)	16 (26.6)	60 (100)
No abortion (controls)	4 (16.6)	20 (83.3)	24 (100)
Total	48 (57.1)	36 (24.8)	84 (100)

Table 3. Frequency distribution of *Toxoplasma gondii* IgG antibody using ELISA test in seropositive women with a history of abortion compared with healthy controls

Type of cases	IgG positive (%)	IgG negative (%)	Total (%)
Single or repeated abortion	18 (56.2)	14 (43.7)	32 (100)
No abortion (controls)	2 (9)	20 (90.9)	22 (100)
Total	20 (37)	34 (62.9)	54 (100)

Table 4. Frequency distribution of *Toxoplasma gondii* IgM antibody using ELISA test in seropositive women with a history of abortion compared with healthy controls

Type of cases	IgM positive (%)	IgM negative (%)	Total (%)
Single or repeated abortion	14 (43.7)	18 (56.2)	32 (100)
No abortion (controls)	2 (9)	20 (90.9)	22 (100)
Total	16 (29.6)	38 (70.3)	54 (100)

Table 5. Frequency distribution of *Toxoplasma gondii* IgG and IgM antibodies using ELISA test in seropositive women with a history of abortion compared with healthy controls

Type of cases	IgG&IgM positive (%)	IgG&IgM negative (%)	Total (%)
Single or repeated abortion	12 (27.2)	32 (72.7)	44 (100)
No abortion (controls)	0 (0)	24 (100)	24 (100)
Total	12 (17.6)	56 (82.3)	68 (100)

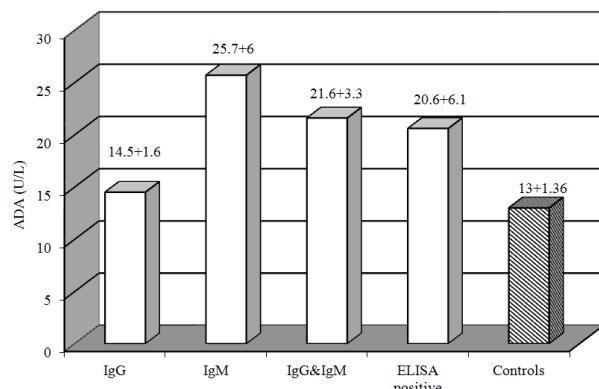


Fig. 1. The mean of Adenosine Deaminase Activity (ADA) in the sera of seropositive women for toxoplasmosis compared with healthy controls

Adenosine Deaminase Activity Result

The mean concentration of ADA in the women who had anti-*Toxoplasma* IgM antibody was significantly higher ($P<0.0001$) than that of healthy controls and the women who had IgG, while there was no significant differences between women who had IgG antibody and healthy controls ($P>0.05$). Figure 1 reveals the mean of ADA level in the women according to the presence and absence of anti-*Toxoplasma* specific antibodies.

Discussion

Since a relationship exists between ADA activity and the cell mediated immune response and because there is no data about ADA activity in toxoplasmosis, it was aimed to determine serum ADA activity of patients with toxoplasmosis and compare the results with the control group. ADA is found in most cells, but its chief role concerns the proliferation and differentiation of lymphocytes especially T lymphocytes (Ozcan *et al.*, 1998).

This study shows increasing levels of ADA in women that had anti-*Toxoplasma* IgM antibody. This result confirms the fact that *Toxoplasma* antigen induces T-lymphocytes proliferation. However, the enzyme activity increases substantially during mitogenic (Galanti *et al.*, 1981) and an antigenic response of lymphocytes and conversely, lymphocyte blastogenesis is inhibited by inhibitors of ADA (Piras *et al.*, 1982). As a marker of cellular immunity, its plasma activity is found to be elevated in those diseases in which there is a cell-mediated immunity (Sullivan *et al.*, 1977; Van der Weyden and Kelley, 1977).

ADA is an enzyme capable of catalyzing purine bases and whose principal biologic activity is detected in T lymphocytes (Hirschhorn, 1990). The role of this enzyme in cellular immune function was highlighted

following the discovery of reduced levels in patients with severe combined immune deficiency (Shubber *et al.*, 1999). Patients with a low level of ADA activity have a later onset of clinical disease owing to a slower and sometimes less complete loss of immune function (Juma *et al.*, 2003).

Similar results of decreased ADA activity in parasitic diseases were observed in schistosomiasis in humans (Juma *et al.*, 2003) and fascioliasis gigantica in sheep (Shubber *et al.*, 1999) and in mice experimentally infected with *Echinococcus granulosus* (Al-Ubaidi *et al.*, 2003). The reduction of ADA level could be related to the state of immune unresponsiveness associated with hydatid disease in humans (Al-Ubaidi *et al.*, 2003).

Another study related with current study showed decreased in ADA level in patients with anti-*Toxolasma* IgG antibody when compared with control group and same results obtained in patients with Giardiasis (Karaman *et al.*, 2009). All these studies revealed that ADA is actually related to the proliferative activity and the differentiation of the lymphocytes (Galanti *et al.*, 1981).

Conclusion

Toxoplasmosis responsible for abortion in infected pregnant women and there must be an evidence sustains the immune tests used in the diagnosis of toxoplasmosis. This study opens up new prospects for use of ADA as an indicator for toxoplasmosis infection.

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Author's Contributions

Ahmed H. AL-Khafajy: Preparation, techniques and publication.

Tarik I. AL-Jeboori: Design the plane.

Ameena S.M. Juma: Design the proposal.

Ethical

This research underwent to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Iraqi Ministry of Health also got the approval of the research by the Committee of ethical standards in the Faculty of Medicine, Al-Nahrain University , one of the colleges affiliated to the Ministry of Higher Education and Scientific Research, Iraq.

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