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High Asymmetric Dimethylarginine (ADMA) Levels in Patients with Brucellosis

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Abstract—Asymmetric dimethylarginine (ADMA) is the main endogenous inhibitor of nitric oxide synthase and is considered to be associated with endothelial dysfunction. Brucellosis, a zoonotic disease caused by *Brucella* spp., can manifest as vasculopathy. The present study was performed to investigate the relationship between ADMA and brucellosis. Serum samples from 39 patients with an accurate diagnosis of brucellosis and from 18 healthy control individuals were included in this study. ADMA levels were significantly higher in the patient group than the controls ($P<0.001$). Receiver operating characteristic (ROC) curve analysis indicated that ADMA level ≥0.61 had a sensitivity of 79.5 %, specificity of 88.9 %, positive predictive value of 93.9 %, and negative predictive value of 66.7 %. This is the first report of an association between brucellosis and high levels of ADMA. In conclusion, ADMA levels should be tested in brucellosis cases and that further studies to clarify the mechanism underlying the association between ADMA and brucellosis are required.

KEY WORDS: asymmetric dimethylarginine; ADMA; brucellosis; *Brucella*.

INTRODUCTION

Nitric oxide (NO) has been reported to be an important mediator of host defense and vascular tone. Asymmetric dimethylarginine (ADMA) is the main endogenous inhibitor of nitric oxide synthase. ADMA regulates nitric oxide formation. The endothelium plays a role in the maintenance of vascular structure and tone and NO, a major endothelium-derived vasoactive mediator. Therefore, ADMA is considered to be associated with endothelial dysfunction. In addition, ADMA level has been shown to be related to hypercholesterolemia, atherosclerosis, hypertension, chronic heart failure, inflammation, and stroke. The relationship between ADMA and infectious diseases has also been investigated, and ADMA levels were shown to be increased in some bacterial infections, HIV, and sepsis and to decrease upon treatment for HIV. ADMA has also been reported to be a predictive marker of mortality in critically ill patients [1, 2].

Brucellosis is a zoonotic disease caused by *Brucella* spp., which is transmitted via milk products. Therefore, this disease is frequently observed in regions in which animal husbandry is common. Brucellosis is an infectious disease characterized by multisystem involvement [3], which can also manifest as vasculopathy. *Brucella* should be considered in the differential diagnosis of vasculitic diseases, particularly in endemic regions [4].

The present study was performed to determine ADMA levels in sera of patients with brucellosis and healthy controls and to investigate the relationship between ADMA and brucellosis.

MATERIALS AND METHODS

Selection of Patients

Patients were diagnosed with brucellosis based on clinical and laboratory findings. The patients fulfilled all

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of the following inclusion criteria: (a) isolation of *Brucella* spp. from bone marrow, blood, or other body fluids, such as cerebrospinal fluid, etc.; (b) positive standard agglutination and/or Coombs tests (titer ≥1/160) in serum and a fourfold rise in titer following the onset of symptoms—the agglutination test was performed using a commercial kit (Cromatest, Krickerbacker Laboratories, Barcelona, Spain; and Refik Saydam Hifzissihha Center Antigene-Antibody Production and Research Laboratories, Ankara, Turkey)—positive serum samples were examined by serial dilution from 1/40 to 1/10,240 if necessary, and cultures were identified using an automated culture identification system (Bactec 9000 Diagnostic System; BD BACTEC Instrumented Blood Culture Systems, Franklin Lakes, NJ or Phoenix Diagnostic Systems, Sparks, MD); (c) a compatible clinical picture, such as arthralgia, fever, sweating, chills, headache, and malaise supported by laboratory findings.

Screening was performed by slide agglutination or Rose Bengal plate agglutination test (Pendik Veterinary Control and Research Institution, Istanbul, Turkey; and Refik Saydam Hifzissihha Center Antigene-Antibody Production and Research Laboratories, Ankara, Turkey).

According to these criteria, a total of 31 acute and eight subacute brucellosis patients were included in the study.

Selection of the Healthy Control Group

The control group consisted of healthy individuals without any symptoms or findings concerning any infectious disease, including brucellosis, and without any history of infection with fever for the previous 6 months. No *Brucella* agglutination test was performed in this group due to a lack of indications.

Sera

Serum samples from 39 patients with an accurate diagnosis of brucellosis were included in this study. Of these, 14 samples were collected from patients admitted to clinics of Abant Izzet Baysal University (AIBU) Faculty of Medicine and the remaining 25 were collected from patients of Yüzüncüyil University Faculty of Medicine. All of the sera had titers higher than 1:160 by Wright agglutination test. Sera from 18 healthy individuals were included as the control group.

Testing of ADMA Levels

ADMA levels were tested by enzyme-linked immunosorbent assay (ELISA) using an ADMA ELISA kit (Immunodiagnostik AG, Bensheim, Germany) at the laboratory of Department of Medical Biochemistry of AIBU Faculty of Medicine. Coefficients of variation were 6.2–7.9 % for inter-assay and 6.2–10.5 % and intra-assay. The sensitivity of the kit was 0.05 μmol/L as described by the manufacturer.

The other variables such as C-reactive protein (CRP), white blood cell count (WBC), red blood cell (RBC), aspartate amino transferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), etc. were tested using the standard laboratory methods and procedures.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 17 (SPSS Inc., Chicago, IL). Continuous variables were tested for normality using the Shapiro–Wilk test. Values are presented as the means ± standard deviation or in the case of non-normally distributed data as medians (interquartile range). The chi-square test was used for intergroup comparisons. The independent samples *t* test or Mann–Whitney *U* test was used for comparison between the two groups. Spearman's rank correlation test was used to determine correlations between the indicated parameters. The capacity of serum ADMA levels for predicting the presence of the disease in patients with brucellosis was determined by receiver operating characteristic (ROC) curve analysis. The sensitivity and specificity were presented when a significant cutoff value was identified. In all analyses, $P<0.05$ was taken to indicate statistical significance.

RESULTS

ADMA levels were significantly higher in the patient group than the controls ($P<0.001$) (Table 1). There were no differences between the groups in terms of age or gender distribution ($P=0.47$ and $P=0.77$, respectively) (Table 1). Table 2 shows the mean ± standard deviation or medians (min–max) of some variables of the sera from the patient group. ROC analysis indicated that ADMA level ≥0.61 μmol/L had a sensitivity of 79.5 %, specificity of 88.9 %, positive predictive value of 93.9 %, negative predictive value of 66.7 %, and accuracy of 82.46 % (area under curve, 0.858; $P<0.001$; lower bound, 0.756; upper bound, 0.959) (Fig. 1). In addition, ADMA was found correlated with age ($R=0.464$, $P=0.005$), ALT ($R=0.540$, $P=0.001$), GGT ($R=0.520$, $P=0.002$), LDH ($R=0.394$, $P=0.023$), and albumin ($R=-0.526$, $P=0.004$) (Table 3).

Table 1. Differences Between Groups in Terms of Age, Sex, and ADMA

Variables	Control	Patient	*P* value
Age (mean year ± SD)	35.76±18.43	36.91±16.52	0.47
Sexuality (male/female)	9/9	19/20	0.77
ADMA-median (min–Max) (μmol/L)	0.44 (0.22–0.67)	0.74 (0.10–2.40)	<0.001*

ADMA asymmetric dimethylarginine; *SD* standard deviation
*$P<0.05$

Significant correlations were found between age and WBC, neutrophil, ALT, AST, GGT, LDH, glucose, urea, creatinine, albumin, and platelet count; between CRP and sedimentation, GGT, glucose, creatinine, and albumin; between sedimentation and albumin; between WBC and neutrophil, lymphocyte, AST, LDH, RBC, platelet count, and hemoglobin. There were no other associations or correlations between other variables, such as CRP, WBC, AST, ALT, GGT, RBC, albumin, glucose, urea, creatinine, prothrombin time, international normalization ratio, hemoglobin, platelet count, sedimentation rate, or age (Table 3).

DISCUSSION

ADMA has recently attracted attention due to its association with many diseases. In some diseases, the association or mechanism of pathogenesis affecting the levels of ADMA have not been clearly identified [1, 2]. In addition, the relationship between ADMA and infectious diseases has not been investigated extensively. In the present study, we demonstrated an association between a high ADMA level and brucellosis.

Brucellosis can cause vasculitis with no predilection of size or location of vascular structure, although arterial and/or venous structures may be involved. Some reported cases with skin lesions include vasculitis of the dermis. Brucellosis can also cause deep venous thrombosis in the extremities, likely due to the compressive effect of a local soft tissue mass or abscess or an immune mechanism, such as a transient induction of autoantibodies and

Table 2. Mean Values of Variables in the Patient Group

Variables	Mean ± SD or median (min–max)
White blood cell ($\times10^3$/μL)	6.80 (3–13.40)
C-reactive protein (mg/L)	30 (3–228)
Sedimentation	25.00 (4–66)
Hemoglobin (mg/dL)	12.80 (6.30–16.50)
Platelet count (/μL)	212,484±105,699
Glucose (mg/dL)	111.19±31.16
Urea (mg/dL)	27.0 (12.0–126.0)
Creatinine (mg/dl)	0.70 (0.40–3.11)
Aspartate amino transferase (U/L)	48.0 (16–702)
Alanine aminotransferase (U/L)	42.0 (14–600)
Albumin (g/dL)	3.70 (2.00–4.60)
International normalization ratio	1.00 (0.87–3.00)
Prothrombin time (seconds)	14.00 (11.20–36.00)
Lactate dehydrogenase (U/L)	540 (231–1567)
Gamma glutamyl transferase (U/L)	70 (9–446)
Alkaline phosphatase (U/L)	310 (100–1291)
Total protein (g/dL)	7.00±4.60
Total bilirubin (mg/dL)	0.60 (0–5.70)
Neutrophil ($\times10^3$/μL)	4.0 (1.0–11.5)
Lymphocyte ($\times10^3$/μL)	2.0 (0.4–5.5)
Red blood cells ($\times10^6$/μL)	4.38±0.71

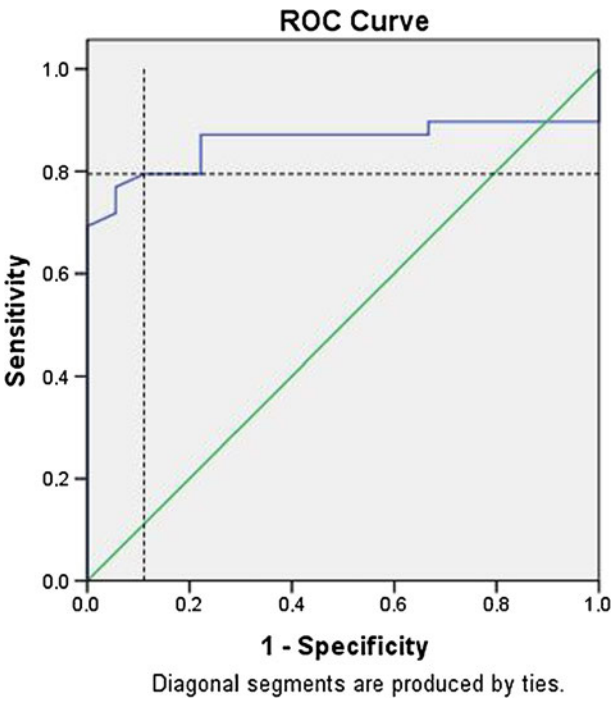


Fig. 1. ADMA level ≥0.61 μmol/L had a sensitivity of 79.5 %, specificity of 88.9 %, positive predictive value of 93.9 %, negative predictive value of 66.7 %, and accuracy of 82.46 % (AUC, 0.858 $P<0.001$; LB, 0.756; UB, 0.959).

Table 3. Correlations of Variables According to Pearson's or Spearman's Test in the Patient Group

	Age		ADMA		C-reactive protein		Sedimentation		White blood cell	
Variables	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value
Age	–	–	0.464**	0.005	0.275	0.121	0.247	0.166	−0.345*	0.049
ADMA	0.464**	0.005	–	–	0.165	0.358	0.321	0.069	−0.174	0.332
C-reactive protein	0.275	0.121	0.165	0.358	–	–	0.573**	<0.001	−0.284	0.109
Sedimentation	0.247	0.166	0.321	0.069	0.573**	<0.001	–	–	−0.264	0.138
White blood cell	−0.345*	0.049	−0.174	0.332	−0.284	0.109	−0.264	0.138	–	–
Neutrophil	−0.365*	0.037	−0.182	0.311	−0.143	0.427	−0.173	0.336	0.775**	<0.001
Lymphocyte	−0.111	0.538	0.244	0.171	−0.323	0.066	0.067	0.713	0.449**	0.009
Alanine amino transferase	0.415*	0.016	0.540**	0.001	0.225	0.208	0.298	0.092	−0.238	0.183
Aspartate amino transferase	0.417*	0.016	0.451**	0.008	0.235	0.188	0.188	0.295	−0.374*	0.032
Alkaline phosphatase	0.236	0.193	0.303	0.092	0.105	0.569	0.252	0.164	0.072	0.695
Gamma glutamyl transferase	0.566**	0.001	0.520**	0.002	0.439*	0.012	0.312	0.083	−0.214	0.240
Lactate dehydrogenase	0.386*	0.026	0.394*	0.023	0.225	0.208	0.115	0.525	−0.613**	<0.001
Glucose	0.560**	0.001	0.235	0.196	0.368*	0.038	0.257	0.156	−0.149	0.417
Urea	0.531**	0.003	0.263	0.160	0.310	0.096	0.154	0.417	−0.280	0.133
Creatinine	0.537**	0.001	0.242	0.175	0.356*	0.042	0.012	0.947	−0.290	0.102
Total protein	−0.114	0.562	−0.108	0.584	−0.288	0.137	−0.043	0.828	0.108	0.584
Albumin	−0.455*	0.015	−0.526**	0.004	−0.468*	0.012	−0.530**	0.004	0.325	0.091
Total bilirubin	0.237	0.185	0.311	0.078	0.203	0.257	0.271	0.127	−0.131	0.468
International normalization ratio	−0.076	0.676	0.142	0.431	−0.042	0.817	−0.016	0.929	0.058	0.749
Prothrombin time	0.016	0.930	0.287	0.105	−0.022	0.902	−0.067	0.712	−0.056	0.756
Red blood cell	−0.013	0.945	−0.059	0.745	−0.101	0.577	−0.106	0.556	0.678**	<0.001
Hemoglobin	0.206	0.250	0.017	0.924	−0.100	0.578	−0.191	0.287	0.421*	0.015
Platelet count	−0.359*	0.040	−0.207	0.248	−0.297	0.093	0.136	0.450	0.405*	0.019

ADMA asymmetric dimethylarginine
*$P<0.05$ significance level; **$P<0.01$ significance level

immune complexes. Brucellosis can cause cerebral vasculopathy [3–5].

Ferrero *et al.* [6] showed that *Brucella abortus* and *Brucella suis* could infect and replicate in human umbilical vein endothelial cells (HUVECs) and in the microvascular endothelial cell line 1 (HMEC-1). Until the study of Ferrero *et al.*, the interaction of *Brucella* with the endothelium had not been characterized, although vascular pathologies, such as vasculitis, endocarditis, and mycotic aneurisms had been described in patients with brucellosis [6]. In brucellosis, the infection is reported to lead to increased production of interleukin (IL) 8 and IL-6 in HUVECs and HMEC-1 cells and increased expression of adhesion molecules. Experiments with purified antigens from the bacterial outer membrane also indicated that lipoproteins, but not lipopolysaccharide, mediate proinflammatory responses. Infected HMEC-1 cells show an increased capacity to promote the transmigration of neutrophils from the apical to the basolateral side of the monolayer, and the same process is observed when live microorganisms stimulate the cells from the basolateral side. These results suggested that *Brucella* spp. can infect and survive within endothelial cells and can induce a proinflammatory response that is likely involved in the vascular manifestations of brucellosis [6].

Dowling *et al.* [7] investigated the effects of ADMA on *Pseudomonas aeruginosa* infection of the respiratory mucosa in nasal turbinate organ cultures. They reported that infected organ cultures had significantly greater epithelial damage and fewer ciliated and non-ciliated cells than the control. They also found that ADMA significantly reduced both bacterially induced epithelial damage and loss of ciliated cells. These findings suggest that NO may be a mediator of the epithelial damage caused by this microorganism.

In a prospective study conducted with intensive care unit patients, O'Dwyer *et al.* [8] reported that the severity of organ failure, inflammation, and presence of early shock in severe sepsis were associated with increased ADMA levels.

In septic shock, upregulation of inducible NO synthase leads to the production of vast amounts of NO, which contributes to pathogen elimination but also to inappropriate vasodilatation and to loss of vascular resistance [1].

ADMA has been implicated as a possible modulator of inducible NO synthase during acute inflammation.

Zoccali *et al.* [9] reported that resolution of acute inflammation was characterized by increased plasma concentrations of ADMA, suggesting that ADMA suppression may stimulate NO synthesis.

Kurz *et al.* [10] found that HIV patients had increased ADMA levels and suggested that increased ADMA production may be related to increased activity of immune activation pathways. In another study, Kurz *et al.* reported a decrease in ADMA levels in HIV patients undergoing antiretroviral therapy [11].

In a study conducted in patients with sepsis, Davis *et al.* [12] suggested that impaired endothelial activity and microvascular function due to increased endothelial NO bioavailability may link plasma ADMA with organ failure and death in sepsis.

In the present study, we found significantly elevated ADMA levels in brucellosis patients. In addition, we found an ADMA cutoff level of 0.61 to have a high sensitivity and specificity. These findings demonstrated the role of ADMA as a marker of this disease.

The role of NO and hence of ADMA in the pathophysiology of inflammation and infection remains controversial. The relationships between ADMA levels and some infectious diseases reported to date support our findings regarding the association with brucellosis. This is the first report of an association between brucellosis and high levels of ADMA. In conclusion, we consider that ADMA levels should be evaluated in brucellosis cases and that further studies to clarify the mechanism underlying the association between ADMA and brucellosis are required.

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The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/ROE8kt.

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