Carbonic anhydrase I and II autoantibodies in Behçet's disease

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ABSTRACT

Background: Behçet's disease (BD) is a vasculitis, seen more frequently around the Mediterranean and the Far East, and evinces with oral and genital ulcerations, skin lesions and uveitis. Carbonic anhydrase (CA) is a metalloenzyme which is widely distributed in the living world, and it is essential for the regulation of acid-base balance. Anti-CA antibodies have been reported in many disorders, such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, endometriosis, idiopathic chronic pancreatitis, type 1 diabetes and Graves' disease. The goal of this study was to investigate CA I and II autoantibodies in BD.

Methods: 35 patients with BD and 29 healthy controls were included in the study and CA I and II autoantibody levels were investigated by ELISA.

Results: The CA I and II autoantibody levels of BD group were significantly higher than the healthy group (p=0.013, p=0.0001, respectively). A cut-off value of 0.250 absorbance unit (ABSU) for anti-CA I was associated with 34% sensitivity and 100% specificity and a cut-off value of 0.171 ABSU for anti-CA II was associated with 54% sensitivity and 100% specificity for predicting BD.

Conclusion: The CA I and II autoantibody levels in patients with BD were found higher compared to control group and the results suggest that CA I and II autoantibodies may be involved in the pathogenesis of BD.

Keywords: Autoantibody; Behçet's disease; Carbonic anhydrase.

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INTRODUCTION

Behçet's disease (BD) is a vasculitis, seen more frequently around the Mediterranean and the Far East, and evinces with oral and genital ulcerations, skin lesions and uveitis[1]. First description of BD has been attributed to Hippocrates, in the "Third book of endemic diseases"[2]. BD was first identified in 1937 by the Turkish dermatologist Hulusi Behçet as a three-symptom complex of recurring oral, genital ulcers and uveitis with hypopyon[3]. The prevalence of BD is reported 80--370/100000 in Turkey, 10/100000 in Japan and 0.6/100000 in Yorkshire[4]. The pathogenesis of BD is poorly understood, and there are many possible mechanisms, such as genetic predisposition, viruses, inflammation, autoimmunity, oxidative stress and toxic agents implicated[5]. There is no specific laboratory test for diagnosis of BD as well as there may be an increase in inflammatory parameters, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), peripheral leukocytes and cytokines including TNF-α, IL-6 and IL-8 during the active phase of the disease[2].

Carbonic anhydrase (CA) is a metalloenzyme which catalyzes the reversible hydration of carbondioxide to bicarbonate and it is essential for the regulation of acid--base balance. CA functions in many physiological and pathological process, such as transport of carbon dioxide, pH regulation, ion transport, formation of stomach acidity, bone resorption and calcification and tumorigenesis are demonstrated. Thus far, 16 isozymes differing from each other with tissue distribution, cell

localization, catalytic activity and resistance to inhibitors, were described[6,7]. In recent years, CA autoantibodies have been demonstrated in some autoimmune, idiopathic diseases and carcinomas, but mechanisms underlying this immune response have not been explained yet[8,9].

The goal of the current study was to investigate CA I and II autoantibodies in subjects with BD and bring a new insight to the autoimmune background of BD.

MATERIAL AND METHODS

STUDY POPULATION AND SAMPLE PREPARATION

Sera were collected from all individuals by a protocol approved by the local ethics committee of the Karadeniz Technical University Medical Faculty. 35 patients with BD, as the study group, and 29 healthy peers, as the control group, were included in this study. Patients were selected from individuals referred from other practitioners to the Physical Medicine, Rehabilitation and Rheumatology Department. Patients were staged according to diagnostic criteria for Behçet's syndrome proposed by the International Study Group[10]. The patients were considered as active if those with oral aphthous ulceration had at least two of the following manifestations present at time of inclusion: genital aphthous ulceration, eye involvement, arthritis, vascular involvement, erythema nodosum, pathergy positivity and high ESR and/or CRP[11]. Patients who had renal, coronary and liver failure, chronic inflammatory diseases, anemia, dyslipidemia, alcohol abuse, and participants who used antilipidemic and antioxidant drugs were excluded from the study. All patients were treated with colchicine and azathioprine.

Blood sample of each subject was collected in vacutainer tubes without anticoagulant. After clotting, samples were centrifuged at 2000 g for 10 min. Serum samples were stored at -80°C until CA I and II autoantibodies measurement.

The following data of the subjects were determined using automatic analyzer: ESR was measured using capillary kinetic photometric assay (Alifax Test1 THL, Polverara, Italy) and CRP was determined using immunoturbidimetric assay (Beckman Coulter AU5800, Mishima, Japan).

DETERMINATION OF SERUM CA I AND II AUTOANTIBODY LEVELS

Specific antibodies to human CA I and II were purchased from Sigma (St. Louis, MO, USA). Serum CA I and II autoantibody levels were determined using enzyme-linked immunosorbent assay (ELISA) according to previously described method[12]. Each sample was assayed in duplicate and the specific binding of serum antibody to CA I or II was calculated as follows: Specific binding = OD_{coated} - $OD_{control}$

STATISTICAL ANALYSIS

Data were shown as mean±standard deviation for normal distributed and median (interquartile range) for non-normal distributed variables. The distribution of CA I and II autoantibody levels in each group were calculated by Kolmogorov-Smirnov test. Comparisons of the Behçet's and control groups were done by Student's t-test for normal distribution and by Mann-Whitney U-test for non-normal distribution. The evaluation of sensitivity, specificity, negative predictive values (NPV) and positive predictive values (PPV) of CA I and II autoantibody in BD group were used by receiver operating characteristic (ROC) curve analysis. Statistical significance was accepted as $p<0.05$.

RESULTS

35 patients with BD and 29 healthy peers were included in this study. In the BD group, 25 patients were in active and 10 patients were in inactive state of the disease. Demographic characteristics of both groups are presented in Table I. The CA I and II autoantibody levels of both groups are shown in Table I. The mean absorbance value of CA I autoantibody for the control subjects was 0.137±0.056 and the absorbance values higher than 0.249, the mean absorbance + 2SD of control subjects, were defined as positive. Positive results were obtained in 12 out of 35 patients with BD (Figure 1). The mean absorbance value of CA I autoantibody of BD group (0.237±0.217) was found to be significantly higher compared with that of the control group (p=0.013) (Table I). The mean absorbance value of CA II autoantibody for the control subjects was 0.107±0.04 and the absorbance values higher than 0.187, the mean absorbance + 2SD of control subjects, were defined as positive. Positive results were obtained in 16 out of 35 patients with BD (Figure 2). The median absorbance value of CA II autoantibody of BD group was found to be significantly higher compared with that of the control group (p=0.0001) (Table I). Also, ESR and CRP values of patients with BD was

TABLE I. DEMOGRAPHIC CHARACTERISTICS AND LABORATORY FINDINGS OF GROUPS

Clinical Characteristics	BD Group (n=35)	Control Group (n=29)	p
Age (year)	36 (35-44)	37 (34-42)	>0.05
Oral aphthous ulceration	% 100	–	
Genital aphthous ulceration	% 80	–	
Eye involvement	% 51	–	
Arthritis	% 54	–	
Vascular involvement	% 37	–	
Erythema nodosum	% 57	–	
Pathergy positivity	% 51	–	
HLA-B5 positivity	% 51	–	
CRP	0.54 (0.32-1.87)	0.16 (0.085-0.25)	0.0001*
ESR	32.1±18.4	8.62±6.89	0.0001**
Anti-CA I Ab	0.237±0.217	0.137±0.056	0.013**
Anti-CA II Ab	0.184 (0.109-0.257)	0.094 (0.071-0.146)	0.0001*

The results were expressed as CRP: mg/dL, ESR: mm/1st hour, anti-CA I Ab: ABSU, anti-CA II Ab: ABSU
Data were expressed as: mean ± SD, median (interquartile range for 25-75%)
**p shows differences between Control and BD according to student t test
*p shows differences between Control and BD according to Mann Whitney U test

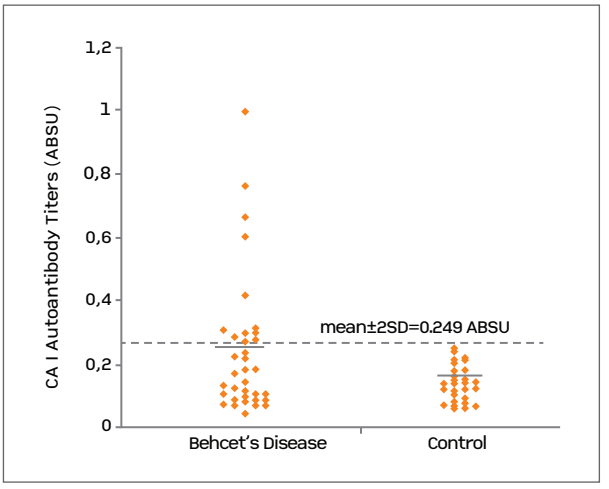

FIGURE 1. The dotted line indicates the mean value plus 2 SD of healthy control sera (A480 = 0.249)

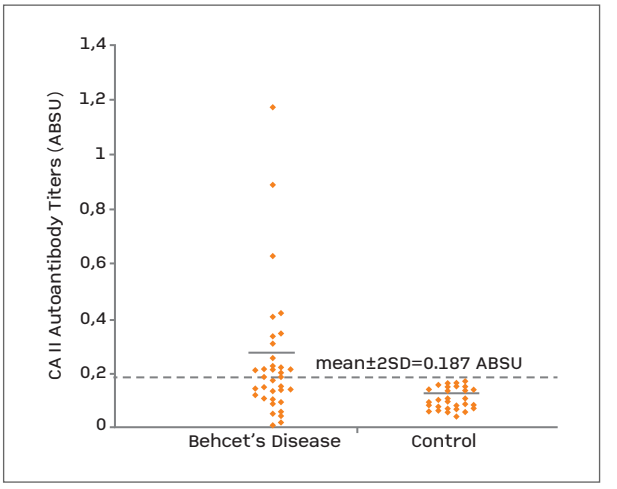

FIGURE 2. The dotted line indicates the mean value plus 2 SD of healthy control sera (A480 = 0.187)

found to be significantly higher than in the control group (p=0.0001) (Table I). CA I and II autoantibodies, CRP and ESR levels were also evaluated with ROC curve analysis. Cut off points, sensitivity %, specificity %, PPV and NPV for these parameters are demonstrated in Table II and Figure 3. Significant positive correlations both between CA I and II autoantibody titers in patients with BD (r=0.972, p=0.0001) and between anti-CA I and ESR titers in patients with BD (r=0.117, p=0.037) were found.

DISCUSSION

BD is a chronic inflammatory disorder and it is characterized by ulcerations, ocular, arthritic, vascular and neurological involvement. There is no specific biochemical parameter for diagnosis of BD. However, CRP, ESR, and leukocyte count are used[13,14]. ESR and CRP levels usually are found higher in patients with BD compared to healthy controls[14,15]. Similar to previous reports, ESR and CRP values of patients with BD were

TABLE II. ACCORDING TO ROC CURVES, SUITABLE CA I AND II AUTOANTIBODIES VALUES AND THEIR SENSITIVITY, SPECIFICITY, PPV AND NPV

	Cut off Point	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV	NPV
CRP	>0.3	89 (73-97)	90 (73-98)	91 (76-98)	87 (69-96)
ESR	>15	89 (73-97)	83 (64-94)	86 (71-95)	86 (67-96)
Anti-CA I	>0.25	34 (19-52)	100 (88-100)	100 (73-100)	56 (41-70)
Anti-CA II	>0.171	54 (36-71)	100 (88-100)	100 (82-100)	64 (49-78)

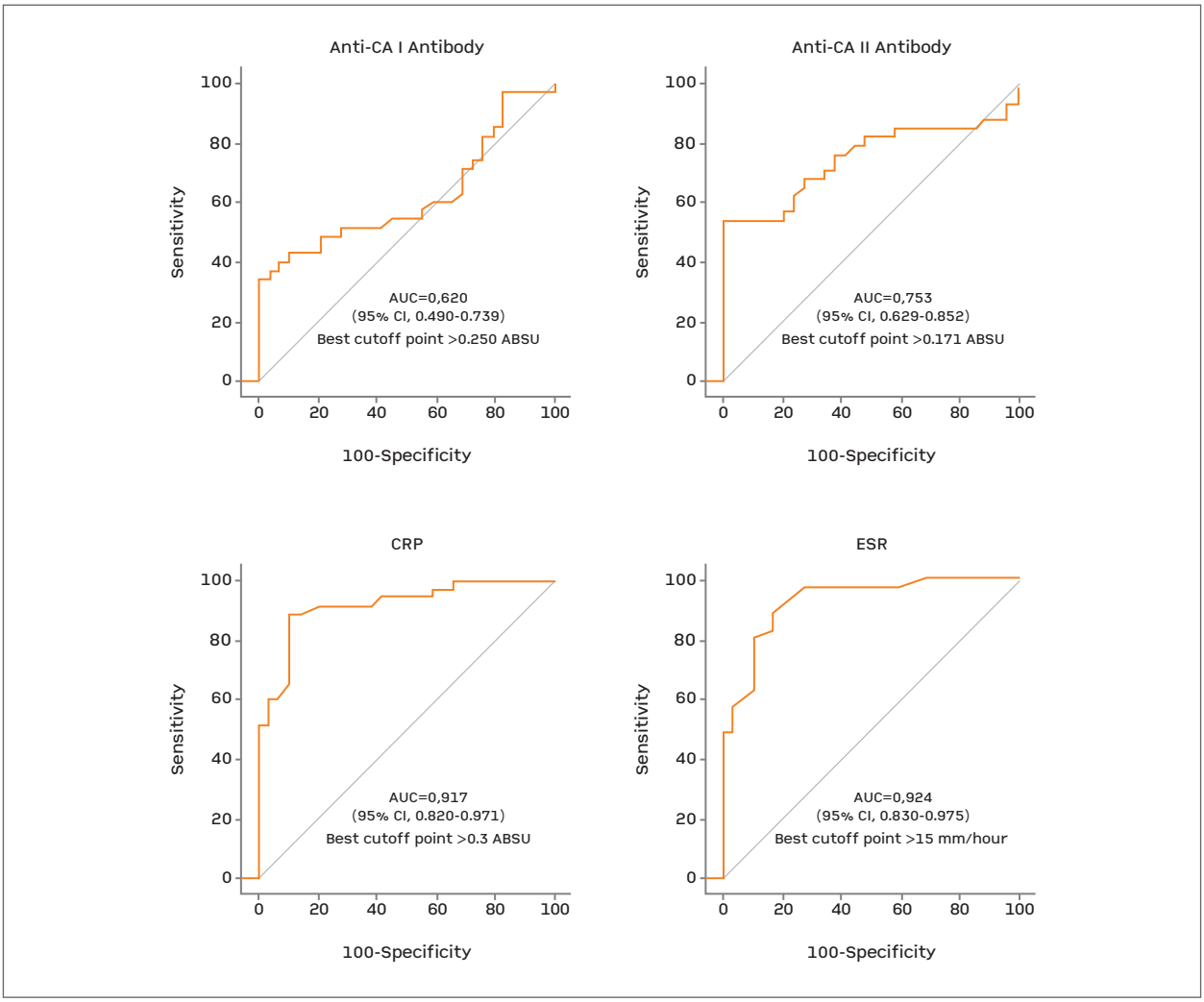

FIGURE 3. ROC curve analysis of anti-CA I antibody; anti-CA II antibody, CRP and ESR values in patient with BD

found significantly higher (p=0.0001) than in the control group in our study. The etiology of BD is unclear, but viruses, autoimmunity, oxidative stress and toxic agent have been implicated[13,14]. Some experts maintain that BD may actually represent one type of autoinflammatory disease, based largely on certain clinical features, including recurrent mucocutaneous lesions and non-deforming arthritis, along with elevated pro-

inflammatory cytokines[1]. Inflammation is one of the main characteristics of BD and it is believed that reactive oxygen species (ROS) are one of the major causes and/or results of this inflammation[3]. Disrupted oxidant/antioxidant balance has been reported in BD patients and it is suggested that this failure may responsible for tissue injury in BD[11].

Carbonic anhydrase isozymes are nearly ubiquitous in living systems and have various functions. CA I and II are the most widely distributed members of the CA family, being present in almost all tissues[16,17]. Recently, autoantibodies against CA I and II were demonstrated in many autoimmune and idiopathic diseases[7,9,12,18-23]. The present study is the first report which shows an increased autoimmune response to both CA I and II in BD patients. We found CA I and II autoantibody prevalence to be 34% and 46%, respectively. As mentioned previously, CA II autoantibodies have been determined in various autoimmune and idiopathic diseases, such as systemic lupus erythematosus (SLE) (28.5%), primary biliary cirrhosis (PBC) (25%), Sjögren's syndrome (SS) (62%), rheumatoid arthritis (27%), endometriosis (70%), type 1 diabetes (65%), Graves' disease (25%), metabolic syndrome (16%) and acute anterior uveitis (15.6%)[9,12,18-25]. Also, CA I autoantibodies have been observed in many diseases, such as Graves' disease (5%), polycystic ovary syndrome (26%) and acute anterior uveitis (20%)[7,12,25]. Also, we determined a significant correlation between CA I and II autoantibody levels in BD patients. This situation may arise from cross-reactivity due to homology of the enzymes. Similar situation is reported in previous reports such as Graves' disease, SLE, SS and PBC[12,21,22]. The high prevalence of CA I autoantibody may indicate an epiphenomenon secondary to the vascular structural changes due to its settlement in vessel and it may have an important role in regulation of vessel tone[24]. CA II is present in every tissue, and autoimmune sialoadenitis was induced in mice by immunization with CA II[16,24]. Thus, it could be a target antigen in the autoimmune diseases related to some tissues or organs. However, increased autoimmunity against these isozymes have been reported in cancer and autoimmune diseases, but no mechanisms have been identified[8]. Iuchi et al demonstrated that superoxide dismutase (SOD)-knock out mouse developed CA II antibodies as a result of increased oxidative stress[26]. 4-Hydroxy-2-Nonenal (HNE) modifies proteins and alters their antigenic properties. Uchida and coworkers reported that CA II is a target for HNE in their study releated with erythrocytes[27]. ROS are physiological activators of many transcription factors such as pro-inflammatory cytokines and adhesion molecules and can play a critical role in apoptosis and autoimmune reactions[28]. The increased ROS and it is result of protein peroxidation have been extensively demonstrated in pathogenesis of many autoimmune diseases[28,29]. We speculate that increased CA antibody titers in BD patients may be a result of increased oxidative damage in these patients.

Autoimmunity against many antigens such as S-antigen, interphotoreceptor retinoid binding protein, heat-shock protein 60, α-enolase, kinectin, α-tropomyosin, heparan sulfate, annexin-V, cardiolipin, selenium binding protein, PTEN-induced putative kinase 1 and switch associated protein 70 are reported in BD and it is believed that these autoantibodies may have a potential diagnostic marker and help in understanding the etiology of BD[30,31]. However, cause of increased autoimmunity against these antigens have not been elucidated clearly[13].

In the present study, CA I and II autoantibody are detected in BD subjects, but the pathogenic role of these antibodies remains uncertain. It shows the need for further studies to evaluate the significance of CA autoantibody production in BD subjects.

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