Evaluation of Endothelial Dysfunction in Bipolar Affective Disorders: Serum Endocan and Urotensin-II Levels

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Objective: This study investigated changes in urotensin-II (U-II) and endocan levels which can be used as an early biological marker of endothelial injury in the episode and remission phases of bipolar affective disorder (BAD).

Methods: We compared endocan and U-II levels, which has been shown to be closely associated with neurotransmitter systems in addition to continuity of endothelial structure and inflammatory response, in patients with BAD in remission for at least one year (n=42) and in patients still in manic or depressive episodes (n=16) with healthy controls (n=30).

Results: Both endocan and U-II levels were significantly higher in the bipolar patients than in the controls. Endocan and U-II levels were also significantly correlated with one another (p=0.000, r=0.833). Both endocan (p=0.000) and U-II levels (p=0.000) were significantly higher in the bipolar attack group compared to the subjects in remission, and in the remission group compared to the controls.

Conclusion: In this study we determined significantly higher endocan and U-II levels in BAD compared to the controls, while serum endocan and U-II levels of patients undergoing attacks were also significantly higher than those of the controls and also those of patients in remission.

KEY WORDS: Endocan; Urotensins; Bipolar disorder; Endothelial injury.

INTRODUCTION

Bipolar affective disorder (BAD) is a chronic disease exhibiting early onset and variation in terms of response to treatment, in addition to combinations of heterogeneous clinical symptoms with an episodic course and a high level of genetic characteristics. It also constitutes a severe psychiatric disease of unclear etiology and course and a serious medical problem representing the 6th largest cause of disability.4)

Oxidative and immunological mechanisms are thought to be involved in many chronic psychiatric and neurodegenerative processes. Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are proinflammatory cytokines most associated with BAD. TNF-α has been shown to remain high after episodes and in intervening periods, while IL-6 is high in both depressive and manic episodes. Studies have reported that less effective monoamine signaling is associated with increased IL-6 levels in cerebrospinal fluid (CSF). Increased IL-6 in CSF in association with recent manic attack in BAD has recently been confirmed. Elevated IL-4, which is thought to be involved in maintaining the balance between cellular and antibody-related immune response, is thought to be more associated with adaptive compensatory mechanisms.5-9)

There is considerable evidence pointing to a relation between BAD and vascular diseases. Adult bipolar patients have an earlier and 5-fold higher risk of cardiovascular disease compared to non-bipolar subjects. Studies of schizophrenia and BAD (and as in one study showing a linear relation between Von Willebrand factor levels and basal ganglion volume) provide evidence for the involvement of inflammation-relayed endothelial
Endocan, also known as endothelial cell-specific molecule 1, is an endothelium-derived proteoglycan capable of binding to numerous bioactive molecules associated with cell signaling, adhesion, proliferation, differentiation and migration, soluble intermediate molecules and the cell surface. It has angiogenic and mitogenic properties and prevents leukocyte migration when inflammation is not present. It is closely associated with immunological response related to inflammation and infection. Its release is increased by vascular endothelial growth factor A (VEGF-A), VEGF-C, IL-1, TNF-α, transforming growth factor β (TGF-β1) and fibroblast growth factor 2 (FGF-2) and reduced by phosphatidylinositol 3-kinase (PI3K) and interferon gamma (INF-γ). It has been reported to be capable of forming as a result of endothelial function with impaired endocan levels. There is increasing suggestion in the literature that serum endocan levels can be used as an early biological marker of endothelial injury, as well as of its probable significance in determining arteriosclerosis and asymptomatic cardiovascular changes in the early stages. In addition, higher endocan levels have been linked to insulin resistance and hypothyroidism.

Urotensin-II (U-II) is a cyclic 11Aa peptide first identified in the caudal neurosecretory system of the teleost fish in 1969. U-II receptors have been determined in the vascular endothelium, myocardium, smooth muscle and skeletal muscles, adrenal glands, thyroid and renal cortex. Although U-II is known as a potent vasoconstrictor, it can exhibit different effects depending on its source, endothelial integrity, vascular size and presence or absence of disease. Elevation has been reported in conditions whose etiology is thought to involve oxidative injury and immunological systems, such as atherosclerosis, endothelial injury, hypertension, diabetes mellitus, obesity, heart failure, kidney failure, and liver diseases. U-II is thought to have diffuse receptor distribution in the central nervous system (CNS), particularly in the cerebral cortex, the olfactory bulb, the pineal gland, and the hypothalamus and to have behavioral and emotional effects in association with neurotransmitter systems, and particularly the dopaminergic, serotonergic, cholinergic and adrenergic systems.

This study investigated changes in U-II and endocan levels in the episode and remission phases of BAD, which can cause endothelial injury despite being a psychiatric disease involving a chronic course and episodes. This will support the previous literature on the subjects of their place in the etiology and monitoring of endothelial injury associated with this disease.

METHODS

We compared endocan levels, a marker of endothelial injury and part of the inflammatory process, and levels of U-II, which like endocan has been shown to be closely associated with neurotransmitter systems in addition to continuity of endothelial structure and inflammatory response, in patients with BAD in remission for at least one year (n=42) and in patients still in manic or depressive episodes (n=16) with healthy controls (n=30). We hypothesized that U-II and endocan, as markers of probable endothelial injury also thought to continue at the cellular level in the euthymic phase, in patients with BAD would differ from levels in the controls.

Patients presenting to our clinics on an in or outpatient basis, aged 18 to 65 years and diagnosed with BAD-I on the basis of diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th edition, text revision and in remission or attack phases and a control group were included in the study after providing written consent. Remission periods were defined as at least one year. Remission was identified on the basis of Hamilton Depression Scale and Young Mania Rating Scale scores of 7 or less. Patients or controls with a history of head trauma, severe metabolic, neurological or systemic disease or with intellectual insufficiency were excluded. Patients' treatments were not managed by the authors, and no intervention took place into their treatment. Treatment and observation were continued as regulated on our ward if the patient was hospitalized or in the clinic if they were treated as outpatients. Outpatients receiving parenteral medication due to excitation were not included in the study until they were able to comply with treatment and progressed to oral drug treatment. If subjects' compliance with treatment was sufficiently impaired as to refuse oral medication, then that consent was not considered valid. However, once they agreed to take medications by the oral route, consent was considered valid.

Our study was completed with 88 subjects, 58 patients with BAD-I and 30 controls. Forty-two patients were in remission and were enrolled from the psychiatric clinic, and
16 were enrolled from patients hospitalized on our ward. The age- and sex-matched control group was selected at random.

Ethical committee approval for the study was granted by the Atatürk University Faculty of Medicine Clinical Research Ethical Committee (No. B30.2.ATA.0.01.00/8 dated 28.22-2014).

**Evaluation Tools**

**Sociodemographic and clinical evaluation form**
Prepared by the author, this form was used to assess not only the participant’s sociodemographic characteristics, but also clinical characteristics such as age at onset of disease, duration of treatment, attack type and number and drugs used.

**Biochemical analysis**
Endocan ELISA kit (Catalogue No. SK00318-06) was purchased from the Aviscera Bioscience Company (Santa Clara, CA, USA). Urotensin-II ELISA kit (Catalogue No. E14913h) was purchased from the EIAab Company (Whuan, China). Biochemistry tubes containing blood specimens collected using the vacuum method from the patient and control groups were left to stand for 20 to 30 minutes at room temperature and away from the light. This allowed fiber networks to form. Specimens were then centrifuged at 3,500 rpm for 10 minutes at +4°C. Each serum specimen obtained was divided into two Eppendorf tubes (1.5 ml) and labeled. We checked that the specimen code on the blood collection tubes matched the number on the Eppendorf tubes. Specimens divided into portions were stored at −80°C until analysis. On the day of study, the specimens in the deep freeze were gradually thawed and investigated once thawing was complete.

**Analysis of Serum Endocan Levels**
Six different concentrations were obtained from the main solution: 100, 50, 25, 12.5; 6.25 and 3.125 ng/ml. Blind and serum specimens were each pipetted into 100-µl wells. Next, 100 µl of detection antibody was added to each well. After being stored at room temperature for 120 minutes, these were aspirated five times. One hundred microliters of horseradish peroxidase conjugate were pipetted into each well and incubated in the dark for 45 minutes. All wells were washed five times. One hundred microliters of substrate solution were added, and the mixture was shaken in a microplate mixer in the dark for 10 minutes. Finally, 100-ml stop solution was added to each well, and the reaction was halted. Blue color proportional to the level of U-II formed as a result of the reaction, and the color change was measured at 450 nm.

**Evaluation of Serum Urotensin-II Levels**
Concentrations of 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, and 0 ng/ml were prepared from the main stock. Blind and serum specimens were each pipetted into 100-µl wells and stored for 120 minutes at 37°C. The fluids were emptied from each well, and 100 µl of detection A reactive was placed into the wells. These were then incubated for 1 hour at 37°C. All wells were washed three times. Next, 100 µl of detection B reactive was placed into each well and incubated for 1 hour at 37°C. These were then again washed three times. In the following step, 100 µl of substrate solution was added and incubated in the dark for 15 minutes. Finally, the reaction was halted by the addition of 100-µl of stop solution.

The end reaction was measured spectrophotometrically on an ELISA reader at a wavelength of 450 nm.

**Statistical Analysis**
Our data were analyzed on IBM SPSS Statistics software (ver. 20; IBM Corp., Armonk, NY, USA). Data were expressed as mean, standard deviation and percentage and evaluated using descriptive analysis. Relation analysis on numerical data was performed using the correlation test, and using the chi-square test for categorical data. Relations among numerical and categorical data were assessed using linear regression analysis. Parametric tests or non-parametric comparisons were selected depending on the sample numbers of the groups to be compared and on homogeneous distribution. Differences between the groups were subjected to post-hoc Tukey test analysis.

**RESULTS**

**Participants’ Demographic Characteristics**
The mean age of the participants was 36.0±9 years. The mean age of the patient group was 36.9±10 years and the mean age of the control group was 34.3±8 years. The ages of the participants ranged between 18 and 56 years. Women constituted 52.3% (n=46) of the participants and
men 47.7% (n=42). Women comprised 55.2% (n=32) of the bipolar group and men comprised 44.8% (n=26). No significant difference was determined in terms of sex between the controls and the bipolar patients. No significant difference was also determined in terms of other demographic characteristics. Smoking levels were 50% (n=15) among the controls and 43.9% (n=25) in bipolar disorder, and no significant difference was determined between the groups. No alcohol and substance use disorder was determined in the control group, but alcohol use disorder was present in three patients with bipolar disorder and substance use disorder in one.

Data concerning psychiatric disease in families were obtained on the basis of information provided by participants. Accordingly, a family history of psychiatric disease was present in 51.7% (n=45) of all subjects, in 57.9% (n=33) of the bipolar disorder group and in 40% (n=12) of the controls. The difference was not statistically significant.

The level of comorbidity with other physical diseases was 16.7% (n=5). Figure for the bipolar group was 42.1% (n=24), and the difference between the groups was statistically significant (χ²=5.724, p=0.017). Degree of functionality, assessed by means of clinical global observation scores, varied significantly between the groups. Although mean values for the control and remission groups were close to one another (100 and 94.8±8.4, respectively), the mean functionality evaluation score in the attack group, 36.0±12.4, was significantly lower compared to the other two groups (p=0.000, f=387.976).

**Evaluation of Clinical Characteristics in Bipolar Disorder**

Of the 58 patients with BAD enrolled in this study, 27.6% were still in the depressive (n=4) or manic episode (n=12), while 72.4% (n=42) were in remission persisting for at least one year. Mean age at onset of disease was 25.4±7.8 and mean duration of disease was 11.9±8.9 years. Mean number of attacks was 4.6±2.2, total number of hospitalizations was 3.4±3.1, mean number of manic episodes was 3.4±2.7, mean number of hypomanic episodes was 3.6±2.5, and mean number of depressive episodes was 2.3±1.7.

The first attack type was manic in 70.7% (n=41) of the bipolar patients and depressive in the remaining 29.3% (n=17). Time between diagnosis and treatment ranged between 1 and 7,200 days. Excluding two patients for whom the available data were inadequate, none of the remaining 56 patients started treatment on the same day that they were diagnosed. In terms of degree of delay, 44.8% (n=26) of patients started treatments within one week, 32.8% (n=19) started treatment after longer than one week but within 100 days, while first treatment took place after more than 100 days in 19% (n=11).

All patients were using medication; 3.4% (n=2) were using lithium bicarbonate alone, 5.2% (n=3) valproic acid alone, 27.6% lithium+antipsychotics (n=16), 37.9% (n=22) valproic acid+antipsychotics, 8.6% (n=5) lithium+valproic acid+antipsychotics, and 17.2% (n=10) antipsychotics alone. Electroconvulsive therapy (ECT) had been applied in the treatment history of 10 (17.2%) of the 58 patients with bipolar disorder, but none were currently receiving ECT.

In terms of the course characteristics of bipolar disorder, seasonality data for eight patients were excluded due to inadequacy of record data and since interviews were not sufficiently reliable. Analysis then revealed seasonality of attacks in 24.1% of patients (n=14), while no seasonality was determined in 58.6% (n=34). Attempted suicide was determined in 20.7% of patients. Since all the female bipolar patients (n=32) had children, postpartum episode characteristics were evaluated in all these subjects, and attacks were determined to be postpartum in onset in 43.8% (n=14) of patients. Attacks triggered by stressors not matching a definition of trauma were present in 44.8% (n=26) of patients, while 31 (53.4%) patients experienced attacks without these being triggered by stressor factors.

**Serum Endocan and U-II Values**

The mean serum endocan level of the participants was 2.34±1.23 and the mean U-II level was 115.72±54.14. Both endocan and U-II levels were significantly higher in the bipolar patients than in the controls. Endocan and U-II levels were also significantly correlated with one another (ρ=0.000 and r=0.833). Both endocan and U-II levels were significantly higher in the bipolar attack group compared to the subjects in remission, and in the remission group compared to the controls (Figs. 1, 2).

When the significant difference between the groups (Table 1) was subjected to post-hoc Tukey test analysis, the significant difference between endocan and U-II val-
Serum Endocan and Urotensin-II Level in Bipolar Disorders

Table 1. Serum endocan and urotensin-II (U-II) levels

| Participant       | Endocan      | U-II         |
|-------------------|--------------|--------------|
| BAD episode (n=16)| 4.65±0.56**  | 196.12±21.48**|
| BAD remission (n=42)| 2.23±0.33**  | 127.22±16.48**|
| Control (n=30)    | 1.22±0.14**  | 53.40±17.19**|
| F                 | 529.97       | 359.57       |

Values are presented as mean±standard deviation.
BAD, bipolar affective disorder.
**p=0.000.

DISCUSSION

Inflammation in an organism, whether in the CNS or not, may be regarded as both positive and negative. It is
thought to initially appear with a healing code, but its long-lasting nature, the fact that it involves numerous inter-related systems and sometimes emerges in an unnecessary or inadequate form can make it difficult to observe its traces and outcomes. However, the immune inflammatory response resulting from adaptation, either initially or during the process involved, is known to lead to injury in the CNS and the periphery. Various consequences, such as neurodegenerative and psychiatric disorders, atherosclerosis, dyslipidemia, insulin resistance and increased mortality may all be observed as a result of this response. 

Proinflammatory cytokines (such as ILs, TNFs, IFNs, TGFs, and chemokines) released from T lymphocytes, macrophages and endothelial cells during inflammation activate neutrophils, cause B cell proliferation, increase vascular permeability and lead to the release of acute phase reactants. Peripheral immune response can extend to the CNS. This can occur as a result of endothelial cells and pericytes in the blood brain barrier (BBB) stimulating inflammatory signals or peripheral immune cells such as T lymphocytes or macrophages passing into the CNS, since cytokines can pass the BBB through active transport, through structures not surrounded by the BBB or by means of afferent vagal fibers such as the nucleus tractus solitarius. Peripheral immune response can extend to the CNS. This can occur as a result of endothelial cells and pericytes in the blood brain barrier (BBB) stimulating inflammatory signals or peripheral immune cells such as T lymphocytes or macrophages passing into the CNS, since cytokines can pass the BBB through active transport, through structures not surrounded by the BBB or by means of afferent vagal fibers such as the nucleus tractus solitarius. There is evidence that inflammation-related endothelial injury plays a role in schizophrenia and BAD. There are studies associating depressive mood with endothelial injury in major depressive disorder and BAD, as well as studies reporting endothelial injury in euthymic BAD patients or even suggesting an association between endothelial injury and hypomanic-manic symptoms, but not depressive symptoms. There are studies showing an increase in endothelial cell activity in BAD and studies...
implicating endothelium-related inflammation.\textsuperscript{45} Endocan and U-II may be involved in endothelial injury associated with inflammatory mechanisms in BAD. Endocan binds to leukocyte ligands that acquire high affinity for E and P secretins during inflammation, and thus becomes significantly involved in inflammation response. Its involvement in this process that supports leukocyte migration is interpreted as preventing leukocyte migration and clustering.\textsuperscript{46,47} Although it appears to prevent migration in conditions in which cell stabilization is not impaired, its activity is rather different after onset of inflammation. While IL-1 and TNF-\(\alpha\) increase the release of endocan, INF-\(\gamma\) and PI3K reduce it. INF-\(\gamma\) inhibits the release of endocan, and when combined with TNF-\(\alpha\) it causes even more powerful endocan mRNA inhibition.\textsuperscript{48,49} U-II has also been shown to be involved in the process in inflammation. It contributes to the release of proangiogenic cytokines from endothelial cells.\textsuperscript{50} It activates monocytes and macrophages and increases the release of IL-1 and IL-6,\textsuperscript{51} and these effects have been shown to be antagonized with U-II receptor blockers.\textsuperscript{52} Due to the anti-inflammatory effect of U-II receptor blockage, it is regarded as a novel therapeutic target for several diseases thought to be based on inflammation.\textsuperscript{53}

Endocan stimulated by proangiogenic factors such as FGF-2 and VEGF becomes involved in vascularization by stimulating types of cells that determine the direction of the vessel and the extent to which it will grow.

Endocan also plays an important role in angiogenesis in stem cells during endothelial-mesenchymal transition.\textsuperscript{54,55} Endocan is although thought to play an important role in arterial medial thickening, plaque formation, and determining the activity zone of endothelial nitric oxide synthase (eNOS).\textsuperscript{56} Thirty-five percent greater nitric oxide (NO) cyclic guanosine monophosphate (cGMP) activity has been determined in tip cells compared to stalk cells that are not associated with vascular growth. Tip cells are known to have a greater endocan release capacity than stalk cells. eNOS/NO/cGMP signaling in tip cells also initiates blood vessel formation.\textsuperscript{57}

Although U-II is regarded as a potent vasoconstrictor, depending on its origin, endothelial integrity, vessel size and presence of disease, it is also capable of performing vasodilation in association with prostaglandin (PG) synthesis, although PGs are not thought to be involved in U-II-mediated vasoconstriction. Vascular tone has a more pronounced vasoconstriction effect in small arteries regulated in an endothelium-dependent manner, although this effect decreases as the vessel diameter increases and the effect in vasodilation associated with smooth muscle functions is almost non-existent. High U-II levels have been determined in patients with atherosclerosis, and U-II predicts a 1.6-fold increased risk for carotid plaque formation.\textsuperscript{58} Intervention in U-II can improve metabolic and atherosclerotic sequelae, and there is also evidence that the use of antagonists or gene deletion can treat atherosclerosis. Inflammatory cells appear to play an important role in U-II-dependent atherosclerosis.

U-II has a proangiogenic effect in endothelial cells in humans and rats and an angiogenic cytokine fibroblast F-like effect and increases mRNA expression of proangiogenic factors. U-II is also thought to activate endothelial proliferation by the P38 P44/42 MAPK pathway.\textsuperscript{59,60} Depending on the binding site, it can also cause an increase in intracellular Ca\(^{++}\) and vasoconstriction, or hyperpolarization and vasodilation by causing the release of NO.\textsuperscript{61} U-II is thought to play a role in both systemic and arterial hypertension, and there may be a positive correlation between CSF U-II concentrations and mean arterial blood pressure.\textsuperscript{62} When U-II binds to its receptor, phospholipase c, activation occurs and inositol triphosphate (IP\(_3\)) and diacylglycerol are formed from PIP2. It subsequently binds to the IP\(_3\) receptor. The Ca\(^{++}\) channels on ER are activated and Ca\(^{++}\) increase. To summarize, U-II increases intracellular Ca\(^{++}\) mobilization. Calcium-related cellular injury is one of the mechanisms discussed in the pathogenesis of BAD. In addition to all the other mechanisms, endocan and U-II are associated with the endocrine system, insulin resistance, obesity and diabetes mellitus. The high comorbidity of metabolic disease and BAD may indicate a common effect.\textsuperscript{63,64}

The distribution of U-II and its receptors in the CNS and their close relation with areas of the brain that regulate neurotransmitters implicated in psychiatric diseases, the locomotor system, the neuroendocrine system and sleep is also worthy of note.

In terms of U-II receptor distribution in the central nervous system, these are found in numerous regions, including the cerebral cortex, olfactory bulb, hippocampus, tegmentum, brain stem, cerebellum and spinal canal.\textsuperscript{65,66} The fact that U-II is present in significant amounts in the locus coeruleus shows that it may have an effect on both
mood and the sleep wakefulness cycle, because U-II increases noradrenaline (NA) release, while its agonists prevent this. U-II affects not only NA, but also the release of neurotransmitters such as dopamine, serotonin and histamine that are known to be involved in several events such as emotional control, sleep, attention, and memory and pain transmission.

Few studies have investigated the relation between serum endocan and U-II levels in psychiatric disorders. One study of subjects with depression in Alzheimer’s disease reported significantly lower endocan levels in subjects with depression and that depressive symptoms decreased as endocan levels increased. This was attributed to the probable effect of cortisol levels that rise in depression on serum endocan levels. Cortisol inhibits endocan gene expression. However, comorbidity of Alzheimer’s and depression is not the same thing as the depressive condition that emerges without Alzheimer’s. For one thing, it must be remembered that they differ in terms of cholinergic reserves. Additionally, the unknown process that initiates plaque formation appears long before clinical forgetfulness appears, and numerous adaptive mechanisms that restore balances for the continuation of connectivity in the nervous system go into operation during that process. Endocan levels may also be affected by inflammatory cytokines associated with depression.

In this study we determined significantly higher endocan levels in BAD compared to the controls, while serum endocan levels of patients undergoing attacks were also significantly higher than those of the controls and also those of patients in remission. In this study, based on the idea that high endocan may be a marker of endothelial injury, such injury was greater in patients with BAD than in the controls, and endothelial injury was also higher in patients currently experiencing manic or depressive attacks compared to patients in long-term remission.

Serum endocan and U-II levels being higher in patients in the attack period compared to those in remission may indicate that endothelial and inflammatory factors underlying the disease persist despite clinical improvement.

The association between time from diagnosis to first treatment and serum endocan and U-II levels, and the significant difference particularly between duration of less than one week and one exceeding 100 days, may be related to endothelial injury persisting throughout the period in the absence of any therapeutic intervention. The length of time in which treatment is delayed, which was significantly correlated with a higher number of depressive episodes, may be associated with a more destructive course of disease.

The significant association in this study between serum endocan elevation and number of depressive attacks, total number of attacks and number of hospitalizations suggests that endothelial injury may be associated with a worse disease course.

Subjects with severe metabolic disease being excluded from the study may be regarded as a limitation. Although, in one respect, this was an advantage since potentially confusing factors were excluded, a potential common biochemical mechanism may perhaps exist between metabolic diseases and bipolar disease, and this could not therefore be identified.

Since the investigation of other comorbid physical conditions in BAD lay outside the scope of this study, our participants did not receive cardiovascular examination, although further studies with larger numbers of subjects may make a significant contribution to the subject. Our subject numbers were not sufficient to reveal the effects of drugs used on serum endocan and U-II levels. In addition, since the drug load calculated on the basis of the levels of drugs used by patients and in the community by years and doses were unknown, we were unable to include the effect of treatment in the analysis.

Further studies with higher sample numbers and supported by blood drug levels are now needed to clarify the effects on the endothelial and inflammatory process of mood regulators and antipsychotics such as lithium and valproic acid.

Although the objectives set out in the methodology were scrupulously adhered to, and the findings were interpreted with the inclusion of confusing factors in the calculations, it is not possible to generalize the results of this study to this disease population.

One of the most powerful aspects of the study is the inclusion of a group with remission for at least one year and an attack group, which is important in revealing biochemical variation in the period in which symptoms do not persist compared to the controls. In addition, this is the first study to evaluate serum endocan and U-II levels in BAD. We therefore think that our study will make a significant contribution to the growing medical literature concerning the role of inflammatory processes and endo-
thelial injury in the etiology and course of BAD.

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