Ischemia modified albumin: a useful marker for increased oxidative stress in Behçet's disease

İskemi modifiye albumin: Behçet hastalığında artmış oksidatif stres için yararlı bir belirteç

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Abstract

Background Increased oxidant stress play an important role in pathogenesis of Behçet's disease (BD). It needs to be clearly defined by using a sensitive marker.

Objective We sought to investigate usefulness of ischemia modified albumin (IMA) to show increased oxidative stress in patients with BD and its value considering the disease activity.

Methods The sera from BD patients (n=57) and healthy individuals (n=45) were collected. IMA, serum total antioxidative capacity (TAC) and total oxidant status (TOS) were measured using Erel's automated method, and the percentage ratio of total peroksit level to TAC level was considered the oxidative stress index (OSI). Receiver operating characteristic (ROC) curves were constructed for all markers.

Results IMA, TAC, TOS, OSI, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels were found to be significantly higher in patients with BD than those in controls. IMA was the only marker which showed difference between active and inactive periods, and it had higher area under curve (AUC) value than those for other markers in ROC analysis (p=0.004). IMA also showed significant correlations with CRP, both in all BD patients and those in active period (r=0.50, p<0.01; r =0.54, p<0.005, respectively).

Conclusions IMA showed superiority to other markers such as TAC, TOS or OSI to evaluate oxidative stress in BD patients as well as in considering disease activity. The higher serum level of IMA and its relationship with CRP observed in active period of BD indicate that IMA may be a useful marker for monitoring disease activity.

Key words: *ischemia-modified albumin, Behçet's disease, oxidative stress, marker*

Özet

Amaç Artmış oksidatif stres Behçet hastalığının patogenezinde önemli bir rol oynar. Buna ilişkin anlamlı bir belirtecin net olarak tanımlanması gereklidir. Behçet hastalarında artmış oksidatif stresin gösterilmesinde ve hastalık aktivitesinin değerlendirilmesinde iskemi modifiye albüminin (İMA) değerini araştırmayı amaçladık.

Yöntem Behçet hastalarından (n = 57) ve sağlıklı bireylerden (n = 45) kan örnekleri toplandı. İMA, serum total antioksidan kapasite (TAC) ve toplam oksidan durumu (TOS), Erel'in otomatik metodu kullanılarak ölçüldü. Toplam peroksit seviyesinin TAC seviyesine oranı, oksidatif stres endeksi (OSI) olarak kabul edildi. Tüm belirteçler için ROC eğrileri oluşturuldu.

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Bulgular İMA, TAC, TOS, OSI, C-reaktif protein (CRP) ve eritrosit sedimantasyon hızı (ESR) düzeylerinin Behçet hastalarında kontrollere göre anlamlı derecede yüksek olduğu saptandı. Aktif ve inaktif dönemler arasında istatiksel olarak anlamlı fark gösteren tek belirteç İMA idi. ROC analizinde diğer belirteçlere göre eğri altındaki alan (AUC) değeri istatiksel olarak anlamlı ölçüde daha yüksek bulundu (p=0.004). İMA, aynı zamanda hem tüm Behçet hastalarında, hem de aktif dönemdeki hastalarda CRP ile anlamlı korelasyon gösterdi (sırasıyla r=0.50, p<0.01; r=0.54, p<0.005).

Sonuç İMA, Behçet hastalarında oksidatif stresi değerlendirmenin yanı sıra hastalık aktivitesini değerlendirmek için de, TAC, TOS veya OSI gibi diğer belirteçlere üstünlük gösterdi. Behçet hastalığının aktif döneminde gözlenen en yüksek İMA düzeyi ve CRP ile ilişkisi, İMA'nın hastalık aktivitesini izlemek için yararlı bir belirteç olabileceğini göstermektedir.

Anahtar kelimeler: *iskemi modifiye albümin, Behçet hastalığı, oksidatif stres, belirteç*

Introduction

Behçet's disease (BD) is a chronic multisystemic vasculitis characterized by attacks of acute inflammation and mostly recurrent oral and genital ulcers, cutaneous, ocular, arthritic, gastrointestinal, neurologic and vascular involvement.[1,2]

BD progresses by unpredictable flares alternating with remission periods. The etiopathogenesis of BD have not been clarified yet. Pathologically, BD is characterized by tissue infiltration of activated polymorphonuclear cells (PMN), and various functions of neutrophils in peripheral blood, such as chemotaxis, phagocytosis, and generation of reactive oxygen species (ROS) increased in BD. ROS-mediated oxidative stress related to neutrophil activation may have an important role in the pathogenesis and severity of BD.[3,4]

Various clinical studies have determined a significant imbalance in the oxidant/antioxidant status in BD, comprised of an exacerbation of the oxidant system and a failure of the antioxidant defences.[5,6] In this respect, an increase of different oxidative stress markers in patients with BD were also shown.[7-9] On the other hand, using different markers on various studies result in a disadvantage for comparison of results of those studies. Therefore, Erel developed rapid, easy, stable, reliable and sensitive assays to evaluate total antioxidative capacity (TAC) and total oxidation status (TOS).[10,11] By using these two tests, he calculated oxidative stress index (OSI), basically define sum of the oxidative stress status. He showed that the described method has high linearity and the results are highly reproducible.[12]

Ischemia-modified albumin (IMA) is the variant form of human serum albumin of which N-terminal end has been altered after it has been exposed to ROS in condition of oxidative stress and/or ischemia.[13] IMA, as a marker for ischemia, is a FDA-approved biomarker that can detect myocardial ischaemia within minutes.[14] In clinical and experimental studies conducted so far, it has been demonstrated that IMA elevates depending on oxidative stress after acute ischemia and returns to normal levels in hours after reperfusion.[15,16] An increasing number of studies have shown that serum IMA level is also elevated in various acute ischaemic conditions such as cerabral infarct, pulmonary infarct, bowel infarct and limb ischaemia.[17] In the view of these studies, IMA is accepted as a marker of oxidative stress and it was determined to be associated with other oxidative stress markers.[18-20] Additionally, in a few studies, increased serum IMA levels are found in BD.[21-23]

In the present study, it was aimed to investigate serum IMA, TOC, TAS, OSI as oxidative stress markers, and CRP and erythrocyte sedimentation rate (ESR) as inflammatory markers by considering whether the value of IMA is a sensitive biochemical marker for representing of increased oxidative stress in patients with BD.

Methods

Study design This case-control study was performed at the department of dermatology, Karadeniz Technical University Faculty of Medicine. The study was approved by the local ethics committee.

Patients The study included 57 patients with BD (27 males and 30 females with a mean age of 33.6) who presented for the first time or were followed at the Department of Dermatology and 45 sex-and age-matched

healthy hospital staff volunteers (22 males and 23 females with a mean age 32.6). All patients with BD fulfilled the criteria of the International Study Group for Behçet's Disease.[24] The patients were divided into two groups according to disease activity. The patients in the active group (n=28) had at least one of the BD symptoms (oral or genital ulcerations, erythema nodosum or other skin findings, eye or vascular involvement, positive pathergy test, neurologic or arthritic involvement) despite treatment and those in the inactive group (n=29) were well controlled with anti-inflammatory therapy. Informed consent was obtained from all participants prior to enroll them into the study.

Sampling and measurement of biochemical parameters
Blood samples were obtained after a 12 hour overnight fast. After the subjects rested 15 min, blood samples were collected into tubes with and without EDTA anticoagulant to obtain serum and plasma. Samples were obtained by low-speed centrifugation at 1500 X g for 15 min at 4°C. To reduce inter-assay variation, samples were stored at –80°C and analyzed at end of the study. C-reactive protein (CRP) was assessed by latex enhanced immunonephelometric method (DADE BEHRING, BN II, GmbH, Marburg, Germany). Erythrocyte sedimentation rate (ESR) by classical Westergren method was determined immediately in whole blood with EDTA (1 mg/ml). These parameters were analyzed using routine laboratory quality control studies. The manufacturers supplied reagents were used in each of the analyzers.

Serum total antioxidative capacity (TAC) and total oxidant status (TOS) were measured using a new automated method developed by Erel.[10,11] Inter-assay CVs were 2.5% and 3.1%, respectively. The percentage ratio of total peroxide level to the TAC level was considered the Oxidative Stress Index (OSI).[12]

IMA level was analyzed by using of rapid and colorimetric method described by Bar-Or et al. This method was based on the detection of reduced cobalt to albumin binding capacity. Following procedure was applied; two hundred µL of patient serum was placed into glass tubes and 50 µL of 0.1% cobalt chloride (Sigma, $CoCl_2.6H_2O$) in H_2O was added. After gentle shaking, the solution was left for 10 minutes in order to ensure sufficient cobalt albumin binding. Fifty microliters of dithiothreitol (DTT) (Sigma, 1.5 mg/ml H_2O) was added as a colorizing agent and the reaction was quenched 2 min later by adding 1.0 mL of 0.9% NaCl. A colorimetric control was prepared for preoperative and postoperative serum samples. For the colorimetric control samples, 50 µL of distilled water was substituted for 50 µL of 1.5 mg/mL DTT. Specimen absorbencies were analyzed at 470 nm by a spectrophotometer (Shimadzu UV1601, Australia). The color of the DTT containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSU). Inter-assay CV was 4.2%.

Statistical analysis All the results are expressed as the mean ± standard deviation. Variables were tested for normal distribution with Kolmogorow-Smirnov test. Comparisons between two groups were performed with Student *t* test and Mann Whitney U test. Pearson and Spearman correlation coefficient were used for calculation of correlations. The receiver operating characteristic (ROC) curve was constructed and area under curve (AUC) was computed for multimarker panels. Statistical significance was defined as $p<0.05$. The SPSS statistical software (SPSS for Windows 13, Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

In BD group, mucocutaneous lesions were the most frequent clinical findings. Oral ulcers were present in all BD cases (100%). Genital ulcerations and skin lesions were found in 91.2% (n=52) and 82.4% (n=47), respectively. The pathergy test was positive in 64.9% (n=37) and ocular involvement was present in 50.8% (n=29) of patients with BD. Articular symptoms and vascular involvement were found in 22.8% (n=13) and 17.5% (n=10), respectively. Three of the patients with BD (3.5%) had neuro-BD. The clinical characteristics of the patients with BD are shown in table 1.

Serum levels of IMA, TAC, TOS, OSI, CRP and ESR were shown in table 2. Regarding the comparison of serum IMA levels of patients with BD and controls, significant

Table 1. Clinical characteristics of active and inactive patients with Behçet's disease

Patient No.	Sex/Age (y)	ROU	RGU	Eye inv.	Skin inv.	Vascular inv.	Pathergy	Other findings
1«	F/26	+	+	-	+	-	+	-
2«	M/33	+	+	+	+	+	-	-
3«	F/39	+	+	+	+	-	+	-
4«	F/36	+	+	+	-	-	+	-
5«	F/44	+	+	-	-	-	+	-
6«	M/39	+	+	+	+	-	+	-
7«	M/53	+	+	+	+	-	-	-
8«	F/22	+	+	-	-	-	+	-
9«	F/18	+	+	+	-	-	+	-
10«	F/39	+	+	-	+	+	-	-
11«	F/22	+	-	-	+	-	+	-
12«	F/33	+	+	-	+	-	+	-
13«	M/42	+	+	-	+	-	+	-
14«	F/33	+	+	-	-	-	+	-
15«	M/47	+	+	+	-	-	-	-
16«	M/28	+	+	+	+	-	-	-
17«	F/29	+	-	+	+	-	-	N
18«	F/36	+	+	+	-	-	-	-
19«	F/23	+	+	+	+	-	-	A
20«	F/26	+	-	-	+	-	+	-
21«	F/46	+	+	-	+	-	-	A
22«	F/32	+	+	-	+	-	-	-
23«	M/27	+	+	-	+	-	-	-
24«	M/30	+	+	+	+	-	+	A
25«	M/22	+	+	-	+	+	+	-
26«	M/36	+	+	+	+	-	-	-
27«	M/19	+	+	-	-	-	+	-
28«	M/34	+	+	+	+	+	-	A
29	M/37	+	+	-	+	-	+	-
30	F/21	+	+	-	-	-	+	-
31	F/20	+	+	-	+	-	+	A
32	M/36	+	+	+	+	-	+	A
33	M/43	+	-	+	+	+	-	-
34	F/54	+	-	+	+	+	+	-
35	F/40	+	+	-	+	+	+	A
36	M/37	+	+	+	+	+	+	A
37	F/29	+	+	-	+	-	-	-
38	F/35	+	+	+	-	-	+	A
39	F/37	+	+	+	+	-	-	-
40	M/38	+	+	-	+	-	+	A
41	F/24	+	+	+	+	+	+	PE
42	F/54	+	+	-	+	-	+	A
43	M/44	+	+	+	+	-	+	-
44	F/44	+	+	-	+	-	+	-
45	M/39	+	+	+	-	-	-	N

Table 1. Continued

Patient No.	Sex/Age (y)	ROU	RGU	Eye inv.	Skin inv.	Vascular inv.	Pathergy	Other findings
46	M/21	+	+	-	+	-	-	-
47	M/25	+	+	+	+	-	+	N
48	F/45	+	+	-	+	+	+	-
49	M/19	+	+	-	+	-	+	-
50	F/35	+	+	-	+	-	+	-
51	M/26	+	+	+	+	-	+	-
52	F/37	+	+	+	+	-	+	-
53	M/44	+	+	+	+	-	+	-
54	F/22	+	+	+	+	-	-	A
55	M/41	+	+	-	+	-	+	-
56	M/27	+	+	+	+	-	+	A
57	M/30	+	+	-	+	-	-	-

+, present ; -, absent ; «, active patients with BD ; inv. , involvement ; A, arthralgia/arthritis ; RGU, recurrent genital ulceration ; ROU, recurrent oral ulceration ; N, neuro-BD ; PE, pulmonary embolism

difference was detected (0.65±0.11 and 0.55±0.12 ABSU, respectively and p=0.0001). Serum IMA levels in the patients with active and inactive periods were 0.69±0.11 and 0.61±0.10 ABSU respectively, and the difference was

Table 2. Serum levels of IMA and other oxidative stress markers and inflammatory parameters in patients with active and inactive Behçet's disease (BD) and healthy controls

	Patients with BD (n=57)	Healthy controls (n=45)	p	Patients with active BD (n=28)	Patients with inactive BD (n=29)	p
IMA, ABSU	0.65±0.11	0.55±0.12	**0.0001**	0.69±0.11	0.61±0.10	**0.004**
TOS, mmol H_2O_2 equivalent/L	16.10±7.10	12.80±2.41	**0.010**	16.90±7.69	15.30±6.49	0.894
TAC, mmol Trolox equivalent/L	0.74±0.13	0.86±0.14	**0.050**	0.74±0.13	0.73±0.12	1.000
Oxidative stress index, AU	2.28±1.23	1.84±0.48	**0.003**	2.43±1.40	2.13±0.97	0.831
CRP, mg/dL	1.39±1.60	0.14±0.09	**0.0001**	1.93±1.89	0.89±1.20	**0.009**
ESR, mm/h	30.20±20.00	9.10±5.10	**0.0001**	41.70±21.34	19.10±11.85	**0.0001**

IMA, ischaemia modified albumin ; ESR, erytrocyte sedimentation rate ; CRP, C-reactive protein ; TAC, total antioxidative capacity ; TOS total oxidative capacity ; OSI, Oxidative Stress Index

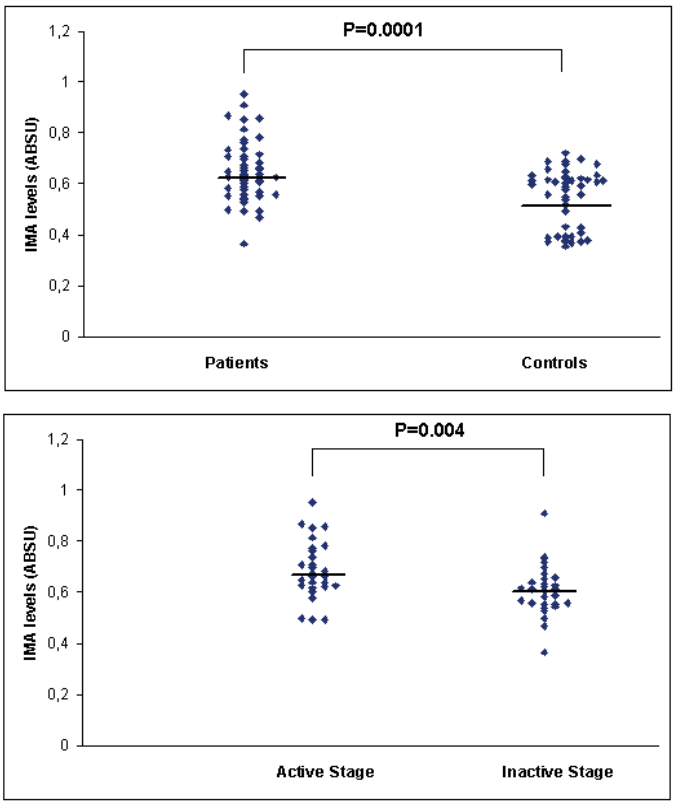

Fig. 1. Scattergram of ischaemia-modified albumin (IMA) levels A) patients with BD and healthy controls B) patients with active BD and inactive BD. ABSU, absorbance units

statistically significant (p=0.004). Scattergram for serum IMA values of patients with BD and controls, and active and inactive patients with BD are shown in figure 1.

There was a statistically significant difference for inflammatory markers, serum ESR and CRP levels, and oxidative stress markers, TAC, TOS and OSI levels as well as IMA between patients with BD and healthy control subjects. However, when compared active and inactive period of the disease, oxidative stress markers, TAC, TOS and OSI, except IMA, CRP and ESR were not found statistically significant.

According to ROC analysis, IMA was found to be higher AUC value than other oxidative stress markers (p<0.004) (Fig. 2).

IMA showed correlations with CRP both in patients with total BD and in active period (r=0.50, p<0.01; r=0.54, p<0.005, respectively) (Fig. 3).

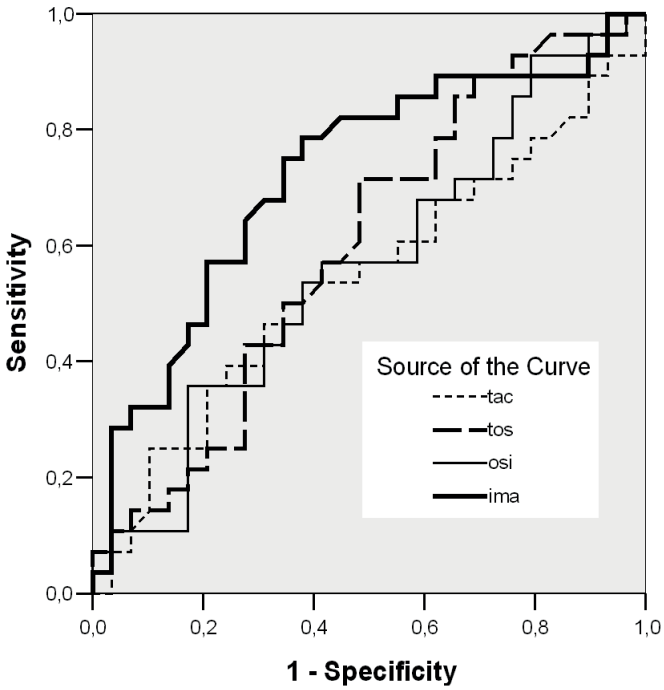

Fig. 2. Receiver operating characteristic (ROC) curves for ischaemia-modified albumin (IMA), oxidative stress index (OSI), total antioxidative capacity (TAC) and total oxidant status (TOS)

	AUC	(95% CI)	p
IMA (ABSU)	0.721	(0.586-0.856)	**0.004**
TOS	0.600	(0.452-0.748)	0.193
OSI	0.564	(0.414-0.715)	0.407
TAC	0.542	(0.389-0.694)	0.587

AUC, Area under curve ; CI, Confidence interval ; IMA, Ischaemia-modified albumin ; OSI, Oxidative stress index ; TAC, Total antioxidative capacity ; TOS, Total oxidant status

Discussion

BD is a chronic inflammatory disease associated with increased oxidative stress. Because of the relationship between increased oxidative stress and pathogenesis of the disease, investigation of a new marker for representing more effectively oxidative stress than current using biomarkers is actively ongoing in research area. In this study, IMA, a well-known FDA-approved sensitive biomarker for myocardial ischaemia, was assessed as a possible biomarker for representing oksidative stress in patient with BD by comparing some other oksidative stress markers and inflammatory markers. Serum

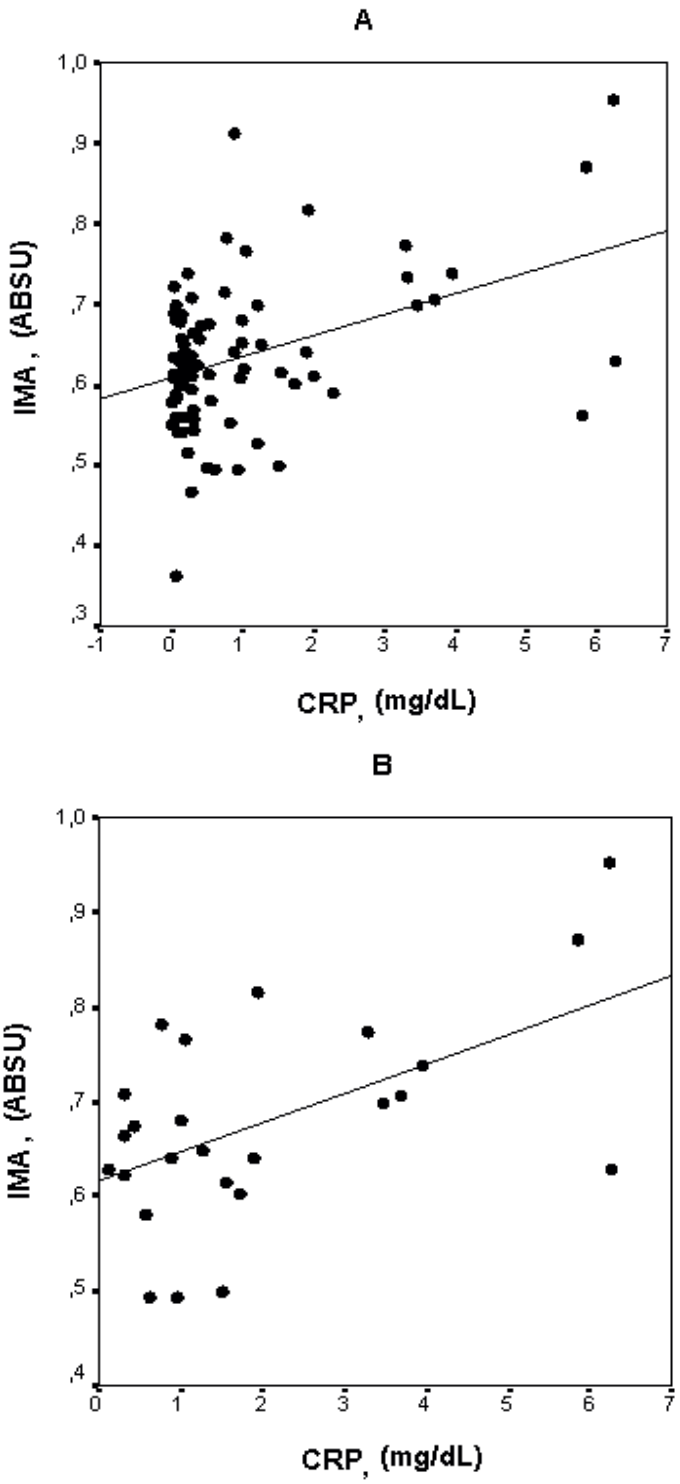

Fig. 3. Relationships between IMA and CRP
A) All patients with Behçet's Disease (r=0.50, p<0.01)
B) Patients with active period (r=0.54, p<0.005)
CRP, C-reactive protein; IMA, Ischaemia-modified albumin

IMA and other markers including TOS and OSI level in total BD patients were found significantly higher from healthy control subjects. As inflammatory markers ESR and CRP levels were also found increased in patients with total BD and active BD. In addition, IMA showed a positive significant correlation with CRP (Fig. 3). These results may be a consequence of the relationship between increased oxidative stress and inflammatory process in BD.

Oxidative stress in BD is a non-specific condition which results from a chronic or active inflammatory state. It is caused by an excess generation of ROS. It was suggested that the vascular and endothelial tissue damage in BD might reflect the increased production of ROS by activated neutrophils.[4,6,7] Whether oxidative stress is the primary triggering factor of endothelium injury in BD or just a non-specific consequence and a marker of inflammation is still debatable. Previous studies confirm that increase of ROS in patients with BD especially in the active period.[5-8,26] Many of these studies tried to demonstrate a new marker for oxidative stress in BD. In this respect, our previous study showed that PMN elastase may be a good biochemical marker for diagnosis and therapy control in patients with Behçet's disease.[27] In our next study, it was tried to describe relationships between pathogenesis and severity of BD and biochemical markers representing oxidant stress and inflammatory condition.[9] Isik et al.[28] reported that OSI value was significantly higher in patient with BD as it was shown in present study. Yazici et al. evaluated advanced oxidation protein products (AOPP) in BD as a new activity marker for oxidative stress.[29] They suggested that AOPP may be a useful marker for monitoring the progress and the severity of the disease activity. Because of relatively early formation of AOPP, greater stability and longer lifespan, protein oxidation products have increasingly been used as a marker in place of lipid peroxidation products in demonstrating oxidative stress.[30] As mentioned above, IMA is a product resulting from oxidative modification of albumin and one of the earliest and sensitive markers for myocardial ischemia.[14]

Regarding Behcet's disease, Omma et al[21] showed sig-

nificantly higher levels of IMA, calprotectin, and hsCRP than the healthy control group in both active and inactive patients. However, although patients with active disease tended to have higher levels of IMA than those with inactive disease, the difference was statistically significant only for hsCRP. In another study reported by Capkin et al[22], serum IMA levels were found significantly higher in patients with vascular involvement in patients with BD. Lastly, Ozyazgan et al[23] reported that serum IMA levels were significantly increased in active periods of patients compared in remission periods of patients with BD and healthy control.

In present study, evaluated oxidative stress markers except IMA did not discriminate oxidative stress status between active and inactive periods of the disease. Therefore, it may be speculated that IMA may be a useful oxidative stress marker representing not only in patients with BD but also in patients with active or inactive periods of the disease. It is well-known that CRP is the most important acute phase reactant using clinical practice in many inflammatory diseases. Observed relationship between IMA and CRP in patients with BD was also continued in active period of the disease (Fig. 3). Therefore, IMA may be also useful during follow-up of the patients. Further large studies on BD patients should be performed to clearly establish the role of IMA in the pathogenesis of the disease.

ROC curve analysis revealed that IMA is more sensitive than other oxidative stress markers (TAC, TOS and OSI) (Fig. 2). ROC analysis gives an opportunity to decide which marker is the best among evaluated markers. IMA showed highest AUC value compared to TAC, TOS and OSI. This means that IMA has superiority from other evaluated oxidative stress markers. There is no information in previous reports evaluating oxidative stress markers by using ROC analysis in BD. Roy et al. firstly showed that in vitro overproduction of ROS can modify the N-terminal region of albumin and increase the concentration of IMA.[13] IMA is a sensitive marker for ischemia. Therefore it elevated in acute ischaemic conditions such as myocardial ischaemia, cerabral infarct, pulmonary infarct, bowel infarct, limb ischaemia.[17] It was also increased in different diseases such as hypercholesterolemia[31], type 2 diabetes[32], obesity[20], and metabolic syndrome.[33] According to Duarte et al.[31] IMA appears to play a role as an oxidative stress biomarker. In view of these studies, IMA was accepted as a marker of oxidative stress and it was determined to be associated with other oxidative stress markers.[18-20]

In conclusion, increased serum IMA level as one of the oxidative stress markers was found in patients with BD and it showed superiority to other oxidative stress markers such as TAC, TOS and OSI for representing oxidative stress status. The higher serum level of IMA and its relationship with CRP observed in the active period of BD indicate that IMA may be a useful marker in monitoring of the disease activity.

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