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Ischemia-modified albumin levels are elevated, and thiol/disulfite homeostasis is impaired in Behçet's disease

Behçet hastalığında iskemi modifiye albümin düzeyleri yükselmiş, tiyol/disülfit homeostazisi ise bozulmuştur

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Abstract

Objective: To investigate the relationship between Behçet's disease (BD) and ischemia-modified albumin (IMA) and thiol molecules and to evaluate serum levels of this molecule in complications that may develop due to BD. BD is a vasculitic disease characterized by recurrent oral starring scarring genital ulcerated lesions and triple complex symptoms of uveitis, histopathologically involving perivascular tissues and vascular wall. It is thought that the mechanism responsible for vascular damage may be immunoregulatory system dysregulation and increased oxidative stress. In this study, serum levels of IMA and native thiol (-SH), total thiol (-SS+-SH), disulfide (-SS) were evaluated in BD and different clinical presentations of the disease.

Methods: Thirty-nine Behçet's patients and 40 healthy volunteers were included in the study. IMA, -SH, and -SS levels were measured using the spectrophotometric (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) method. Statistical analysis were performed using the SPSS version 21.0 package program.

Results: In our study, the mean IMA (p<0.001), -SS/-SH (p<0.01), and -SS/(-SS + -SH) ratios (p<0.01) were found to be significantly higher in Behçet's patients compared to the control group. In receiver operating characteristic (ROC) analysis, the highest associated value with BD was found in IMA (p:0.001, AUC:0.713). In BD, no significant difference was found between those with mucocutaneous involvement and those with organ involvement in terms of IMA and thiol/disulfide parameters (p>0.05).

Conclusion: In our study, we found that IMA serum levels were high, and -SS/-SH and -SS/(-SS + -SH) ratios were significantly low in BD.

Keywords: Behçet's disease, thiol/disulfide homeostasis, IMA

Öz

Amaç: Behçet hastalığı (BH) ile iskemi modifiye albümin (İMA) ve tiyol molekülleri arasındaki ilişkiyi araştırmak ve BH'ye bağlı gelişebilecek komplikasyonlarda bu moleküllerin serum düzeylerini değerlendirmektir. BH rekürren oral aft, skar bırakan genital ülsere lezyonlar ve üveitin üçlü kompleks semptomları ile karakterize, histopatolojik olarak perivasküler dokuları ve vasküler duvarı tutan vaskülitik bir hastalıktır. Vasküler hasardan sorumlu mekanizmanın immünoregülatör sistem disregülasyonu ve oksidatif stres artışının olabileceği düşünülmektedir. Bu çalışmada BH'de ve hastalığın farklı klinik prezentasyonlarında İMA, native thiol (-SH), total thiol (-SS+-SH) ve disülfit (-SS) serum düzeyleri değerlendirilmiştir.

Yöntem: Çalışmaya 39 Behçet hastası ve 40 sağlıklı gönüllü dahil edilmiştir. İMA, -SH ve -SS düzeyleri spektrofotometrik (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) yöntemle ölçülmüştür. İstatistiksel analizler SPSS versiyon 21.0 paket programı kullanılarak yapılmıştır.

Bulgular: Çalışmamızda Behçet hastalarında kontrol grubuna göre İMA ortalaması (p<0,001), -SS/-SH (p<0,01) ve -SS/(-SS + -SH) oranları (p<0,01) anlamlı olarak yüksek bulunmuştur. -SH (p<0,001), -SS +-SH (p<0,001) plazma seviyeleri ve -SH/İMA (p<0,01), (-SS + -SH)/İMA (p<0,01) oranlarının ise Behçet hastalarında kontrollere göre önemli düzeyde daha düşük olduğu tespit edilmiştir. Alıcı işlem karakteristiği [Receiver Operating Characteristic (ROC)] BH ile en yüksek ilişkili değerin İMA'da olduğu bulunmuştur (p:0,001, AUC: 0,713). Behçet hastalarında farklı klinik prezentasyonlar (vasküler, oküler, nörobehçet, mukokutenöz) ile çalışma parametleri arasında yapılan çoklu anova testlerinde anlamlı fark saptanmamıştır (p>0,05).

Sonuç: Çalışmamızda BH'de İMA serum seviyeleri ile -SS/-SH ve -SS/(-SS + -SH) oranlarının istatistiksel olarak anlamlı olacak şekilde hastalıkla ilişkili olduğu tespit edilmiştir.

Anahtar Kelimeler: Behçet hastalığı, tiyol/disülfit homeostazisi, İMA

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Introduction

Behçet's disease (BD) is a variable vascular vasculitis that is characterized by non-scarring recurrent oral starring scarring genital ulcers, histopathological involvement of the vascular wall and perivascular tissues.[1] Although the mechanisms causing vascular involvement have not been elucidated, it is primarily characterized by autoinflammation of the vascular wall. It is thought that immunoregulatory system dysregulation and increased oxidative stress may be associated with the development of vascular damage.[2] Bacterial and viral infections, dysregulations in humoral and/or cellular immunity are thought to affect the etiopathogenesis of the disease with genetic factors. The role of the *HLA-B51* gene, which is positive in nearly 60% of Behçet's patients, in the development of the disease is not fully known.[3,4] Since there are no specific diagnostic biochemical tests and histopathological indicators, the diagnosis of the disease is made according to clinical findings. The time required for a definitive diagnosis can usually be extended up to several years after the first symptoms appear. The prolongation of this period is due to the inconsistency of clinical findings among patients, and the prognosis of the disease depending on geographical, ethnic, and personal differences.[5] Available biomarkers in the diagnosis of BD are not yet sufficient for diagnosis and cannot predict the course of the disease and the response to treatment. There are 585 amino acids in the structure of human plasma albumin. Under physiological conditions, any metal element (cobalt, nickel, copper) can be attached to the N-terminus of the first 3 amino acids in the structure of albumin. Metal-binding capacity may vary depending on hypoxic-ischemic conditions. The form formed by hypoxia events that change the structure of serum albumin is called ischemia-modified albumin (IMA). Ischemia-reperfusion injury increases plasma IMA levels.[6,7] IMA is accepted as a sensitive biomarker as an indicator of ischemia-induced myocardial ischemia in acute coronary syndrome.[8] Additionally, increased IMA plasma levels have been shown in other diseases associated with increased oxidative stress, such as psoriasis, rheumatoid arthritis, and inflammatory bowel diseases.[9-12]

The increase in reactive oxygen species (ROS), which occurs during cellular metabolism such as sueroxide radicals, hydroxyl, and hyhydrogeneroxide, and the deterioration of the oxidative balance due to the inadequacy of the antioxidant level responsible for their neutralization causes the development of oxidative stress. The increase in ROS damages the double bond-containing areas of intracellular proteins and lipids and the double bonds of bases in the structure of DNA. Oxidative stress causes chain oxidation reactions by breaking a hydrogen atom from the double bonds. As a result, cell injury and cellular death occur due to damage to molecular structures such as intracellular proteins, lipids, and DNA.[13] In previous studies in rheumatological diseases, an increase in plasma levels of oxidant radicals secondary to autoimmunity and inflammation was found.[14,15] Similarly, it has been shown that increased oxidative stress also affects the pathophysiology of BD, and the antioxidant level decreases in the plasma of patients.[16] Thiols are one of the main reducing molecules in the human body. ROS produced in various events in the organism are converted into oxidized forms by transferring the excess electrons in their body to thiols. Thus, reversible disulfide bonds are formed. These bonds can be converted back to their old thiol forms when necessary to maintain oxidant-antioxidant hemostasis in the organism. This cycle, which is defined as dynamic thiol-disulfide homeostasis, has important roles in many events such as antioxidant protection mechanism, enzymatic activation, apoptosis, and intracellular signal transduction.[17] Thiol disulfide balance also changes in hepatic damage, cardiovascular events, central nervous system diseases, diabetes mellitus, malignancy, advanced age, and complications related to pregnancy.[18]

Although IMA levels or thiol-disulfide homeostasis have been evaluated alone in different studies in BD, the relationship of these two parameters with each other and with the complications that may develop due to the disease has not been evaluated before. This study was conducted to investigate the relationship between BD and IMA and thiol molecules and to evaluate serum levels of this molecule in complications that may develop due to BD.

Materials and Methods

Patients

A total of 39 Behçet's patients (mean age: 38.74±9.30 years) consisting of 13 females and 26 males, diagnosed according to the 1990 Behçet's Disease International Study Group criteria, followed in the rheumatology department of Ankara City Hospital, and for the control group, 40 healthy volunteers (mean age: 38.8±9.8 years) consisting of 14 females, and 26 males were included. Grouping according to clinical organ involvement patterns in Behçet's patients was made retrospectively from file scanning.

Obtaining Sample Samples and Calculating IMA and Thiol-Disulfide Values

Venous blood samples were taken into approximately 10 mL vacuum tubes and centrifuged at 1.300 g x for 10 min. The aliquoted sera were stored in Eppendorf tubes at -80 °C until the time of analysis. Thiol/disulfide homeostasis parameters were calculated using the automatic spectrophotometric method described by Erel and Neselioglu.[18] In this method,

first, sodium borohydride and disulfide bonds were reduced to free functional thiol groups. The reduced and native thiol (-SH) groups were calculated after the reaction with DTNB [5,5'-dithiobis-(2 nitrobenzoic) acid]. Half of the difference between the total thiols and -SH provides the dynamic disulfide amount. After the determination of disulfide (-SS) and -SH, total thiols (-SH+-SS) amounts, disulfide/native thiol percent ratios (-SS/-SH), disulphide/total thiol percent ratios [-SS/(-SH+ SS)] and native thiol/total thiol percent ratios [-SH/(-SH+-SS)] calculated. To calculate IMA from venous blood samples, the samples were left at room temperature for half an hour and then centrifuged at 3.500 rpm for 5 min. Samples transferred to Eppendorf tubes in aliquots were stored at -80 ºC until analysis. IMA level was measured using the Albumin Cobalt Binding test. This test was performed by mixing the patient's serum with 50 mL of 0.1% cobalt (II) chloride ($CoCl_2$,$6H_2O$) solution (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany). After 10 min of incubation, 50 mL of 1.5 mg/mL dithiothreitol was added to the mixture to induce cobalt binding to the albumin. After a further 2 min incubation, 1.0 mL of a 0.9% sodium chloride solution was added to the mixture to measure the binding capacity. Absorbance determination from the samples was performed using a spectrophotometer at 470 nm. The data obtained are shown as absorbance units (ABSU). After measuring -SS, -SH and -SS + -SH levels, -SS/-SH, -SS/(-SH + -SS) and -SH/(-SH + -SS) percentage ratios were calculated.

Statistical Analysis

Kolmogorov-Smirnov test was used to determine the normal distribution of continuous variables. Independent sample t-tests and Mann-Whitney U test were used to evaluate the presence of a statistically significant difference between the patient and control groups. Pearson correlation analysis was used between parameters in the analysis of correlations. The variables' parametric and non-parametric statistical results are shown as mean ± standard deviation and median (minimum-maximum), respectively. The statistical analyses were performed using the Statistical Packages for the Social Sciences (SPSS) version 22.0 package program. The $p<0.05$ level was taken as the lower limit that was considered statistically significant. While testing the diagnostic accuracy measures of the indexes, receiver operating characteristic (ROC) analysis was used and area under the curve (AUC) was presented with 95% confidence intervals. Youden's index was used while determining the optimum cut-off value and diagnostic accuracy criteria for the cut-off value were presented.

Ethics Approval

The research protocol was approved by the Ankara Yıldırım Beyazıt University Faculty of Medicine Research Ethics Committee (approval number: 1613, date: 14.04.2021) and all patients gave informed written consent to participation in the study.

Patient Consent for Publication

Not required.

Results

Patients with Behçet's disease (n=39), 13 female and 26 male, SS and 40 healthy volunteers, 14 females, and 26 male, were included in the study ($p>0.05$). The mean age of Behçet's patients was 38.7±9.3 years, and the mean age of the control group was 38.8±9.8 years ($p>0.05$). Smoking, body mass index, and presence of comorbid diseases were found to be similar between Behçet's and control groups.

The patterns of organ involvement in the Behçet group and the data on the medical treatments used are shown in Table 1.

The relationship between drugs used with -SH, -SH+-SS, -SS, IMA levels, and -SS /-SH, -SS /(-SH+ SS) values were evaluated with multiple variance analysis ANOVA Post-hoc tests test. No statistically significant difference was observed between the different types of treatments used in Behçet's and these parameters ($p>0.05$). In the evaluation of study parameters in different organs involvement in BD, no statistically significant difference was found between clinical patterns and these parameters ($p>0.05$).

There was no significant difference in study parameters between those only mucocutaneous involvement and those organ involvement in BD (Table 2).

Table 1. Data on organ involvement patterns and medical treatments used in the Behçet group

Parameters	**n (%)**
Mucocutaneous Behçet	13 (33.3)
Neurobehçet	2 (5.1)
Vascular Behçet	13 (33.3)
Ocular Behçet	6 (15.3)
Vascular and ocular Behçet	5 (12.8)
Medical therapy	**n (%)**
Colchicine	36 (82.3)
Corticosteroids	17 (43.5)
Azathioprine	19 (48.7)
Anti-TNF	5 (12.8)
Cyclosporine	2 (5.1)
Cyclophosphamide	3 (7.6)
Interferon	2 (5.1)

Anti-TNF: Anti-tumor necrosis factor

Data on the comparison of study parameters between Behçet's and control groups are shown in Table 3. In the Behçet group -SH (488.77±46.68), -SH+-SS (549.95±69.77), -SH/IMA (511.07±67.13) and (-SH+-SS)/IMA (549.95±69.77) levels compared to the control group [-SH (529.67±55.96), -SH+-SS (629.59±104.65), -SH/IMA (589.76±98.88), (-SH+-SS)/IMA (629.59±104.65)] were found to be significantly low (p<0.001). The rates of -SD/-SH (3.84±0.87) and -SD/(-SH+-SD) (3.55±0.75) in Behçet's patients compared to the control group [-SD/-SH (3.39±0.81), -SS/(-SH+-SS) (3.16±0.71)] was found to be significantly high, while the mean of -SH/(-SH+-SS) was found to be significantly lower (p<0.01). When compared according to IMA levels, the mean of IMA was found to be significantly higher in the Behçet group (0.96±0.06) than in the control group (0.90±0.06) (p<0.001). There was no significant difference between the groups in terms of white blood cells, C-reactive protein, erythrocyte sedimentation rate (ESR), -SS (p>0.05). Figure 1 shows the distribution of IMA, s and Figure 2 shows the distribution of -SS/-SH, SS / (-SH+-SS) levels among the groups.

Table 4 shows the ROC analysis results of -SS/-SH, -SS/(-SH+-S), and IMA levels in BD. The highest value associated with BD was observed in IMA (p=0.001). When a cut-off value of 0.99 was taken for IMA in BD, it was determined that the test gave a sensitive confidence interval of 30.8% sensitivity and 97.5% specificity (AUC=0.713) Figure 3. shows the ROC analysis graph of the operating parameters in BD.

Table 5 shows the correlation between the study parameters. It has been determined that there is a statistically significant negative correlation between IMA and -SH (r:-0,504, p<0.01), -SH+-SS (r:-0,532, p<0.01) ve -SS (r:-0,249, p<0.05) levels. It was determined that there was a statistically significant positive correlation between IMA and CRP (r:0,238, p<0.05) levels.

Discussion

The etiology of BD, which is a systemic vasculitis, is not yet known. The increase in oxidative stress is thought to be an important factor in vascular injury. Stimulated neutrophils produce oxygen intermediates that cause auto-

Table 2. Comparison of patients with only mucocutaneous involvement and those with organ involvement in terms of study parameters in Behçet's patients

Parameters	Only mucocutaneous (n=13)	Organ involvement (n=26)	p-value
-SH, mean ± SD [μmol/L]	494.89±51.09	484.82±46.50	<0.05
-SH+-SS, mean ± SD [μmol/L]	530.14±48.50	523.80±48.47	<0.05
-SH/IMA, mean ± SD [%]	513.37±65.98	508.59±66.72	<0.05
(-SH+-SS)/IMA, mean ± SD [%]	550.05±66.79	549.51±71.07	<0.05

All values were expressed as mean ± SD. All values were calculated using the independent samt-test test for normal distribution. -SS: Disulphide -SH+-SS: Total Thiol, IMA: Ischemia Modified Albumin

Table 3. Comparison of study parameters between Behçet and control groups

Parameter	Behçet	Control	p-value
-SH, mean ± SD [μmol/L]	488.77±46.68	529.67±55.96	**<0.001**
-SH+-SS, mean ± SD [μmol/L]	549.95±69.77	629.59±104.65	**<0.001**
-SS, mean ± SD [μmol/L]	18.58±3.81	17.85±4.17	>0.05
-SS/-SH, mean ± SD [%]	3.84±0.87	3.39±0.81	**<0.01**
-SS /(-SH+-SS), mean ± SD [%]	3.55±0.75	3.16±0.71	**<0.01**
-SH/(-SH+-SS), mean ± SD [%]	92.88±1.51	93.66±1.42	**<0.01**
IMA, mean ± SD [ABSU]	0.96±0.06	0.90±0.06	**<0.001**
-SH/IMA, mean ± SD [%]	511.07±67.13	589.76±98.88	**<0.001**
(-SH+-SS)/IMA, mean ± SD [%]	549.95±69.77	629.59±104.65	**<0.001**
CRP mean ± SD [mg/dL]	0,007±0,0018	0.002±0.001	>0.05
ESR mean ± SD [mm/h]	11.20±7.32	10.025±5.74	>0.05
WBC, mean ± SD [x10^9/L]	7.23±1.89	6.59±1.23	>0.05
Hemoglobin, mean ± SD [x10^9/L]	13.73±1.94	14.19±2.84	>0.05
Creatinine mean ± SD [mg/dL]	0.78±0.16	0,43±0,15	>0.05
ALT mean ± SD [mg/dL]	35.02±15.52	27.30 ±13.46	>0.05

All values were expressed as mean ± SD. All values were calculated using the independent sample t-tests for normal distribution. -SS: Disulphide -SH+-SS: Total thiol, -SH: Native Thiol, IMA: Ischemia modified albumin, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, WBC: White blood cell, ALT: Alanine aminotransferase

Table 4. Specificity, sensitivity, and the cut-off levels of study parameters in Behçet.

	Cut-off	AUC (95% CI)	Sensitivity (%)	Specificity (%)	LR	p-value
-SH	524.2	0.313 (0.197-0.430)	30.8	62.5	0.82	0.004
-SH+-SS	518.7	0.329 (0.211-0.446)	61.5	72	0.82	0.009
-SS/-SH (%)	4.85	0.679 (0.560-0.798)	69.2	67.5	**2.13**	**0.006**
-SS/(-SH+-SS) (%)	4.42	0.679 (0.560-0.798)	69.2	67.5	**2.13**	**0.006**
-SH\(-SH+-SS) (%)	96.26	0.321 (0.202-0.440)	69.2	17.5	0.89	0.006
-SH\IMA (%)	534.53	0.381 (0.170-0.393)	43.6	45	0.79	0.001
(-SH+-SS)\IMA (%)	557.44	0.295 (0.181-0.408)	53.8	33.5	0.79	0.002
IMA	0.99	0.713 (0.600-0.825)	30.8	97.5	**4.10**	**0.001**

AUC: Area under the Curve, CI: Confidence interval, LR: Likelihood ratio. -SS: Disulphide -SH+-SS: Total thiol, -SH: Native thiol, IMA: Ischemia modified albumin

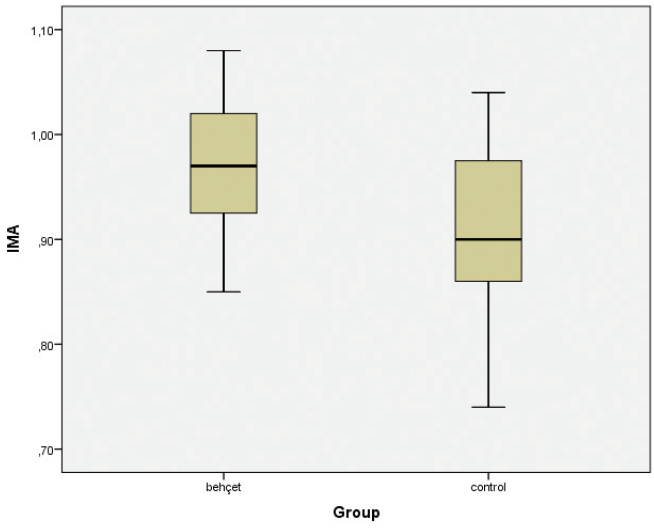

Figure 1. Showing the mean of IMA between the groups
IMA: Ischemia modified albumin

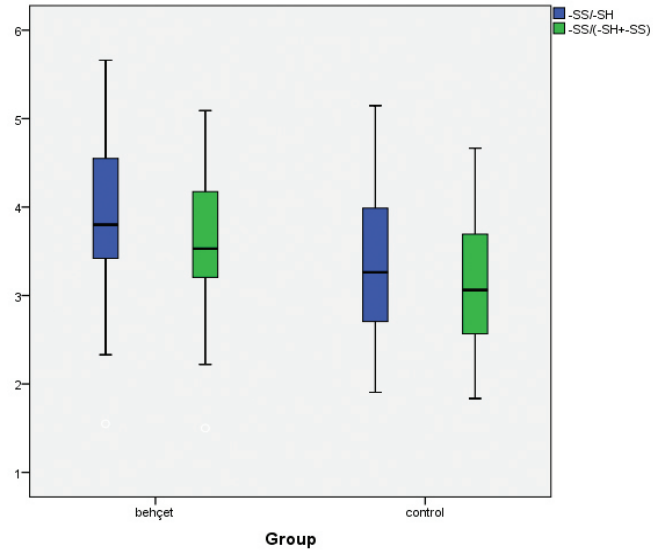

Figure 2. Showing the mean of -SS/-SH and -SS/(-SH+-SH) between the groups
-SS: Disulphide, -SH+-SS: Total thiol, -SH: Native thiol

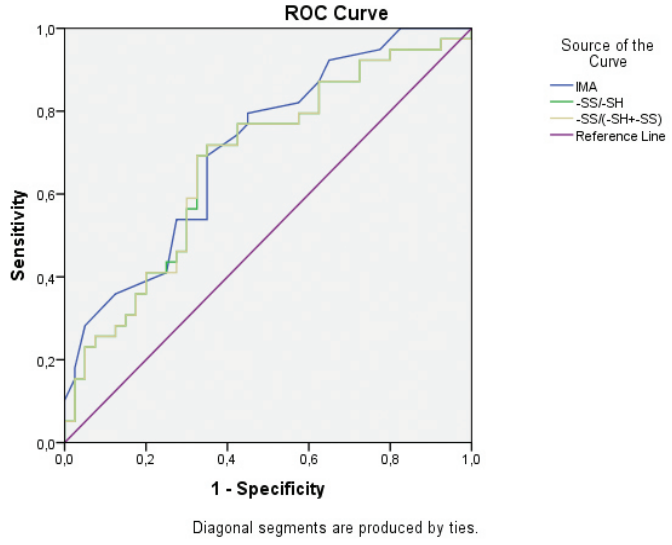

Figure 3. ROC curves of thiol-disulfide homeostasis in Behçet
-SS: Disulphide, -SH+-SS: Total thiol, -SH: Native thiol, IMA: Ischemia modified albumin

oxidative tissue damage.[19,20] It has been shown that there is an increase in oxidative stress due to inflammatory events and that the main cell involved in this increase is activated neutrophils.[21] Similarly, an increase in oxidative stress was found in BD due to excessive ROS production and a decrease in antioxidant levels.[22]

The increase in ROS resulting from the increase in oxidative stress causes many chemical changes in the albumin structure and causes the formation of IMA. It has been stated that IMA may be a new biomarker in increased oxidative stress and ischemia, and it has been shown that plasma IMA levels increase during oxidative events.[23] However, it has been determined that thiol and disulfide molecules form an important antioxidant defense system during oxidative reactions, and their plasma levels increase in case of increased oxidative stress.[24] It has been reported that deterioration in the oxidant-antioxidant balance in the body affects the emergence of various diseases.[25-27]

Table 5. Correlation between study parameters

Parameter	-SH	-SH+-SS	-SS	IMA	CRP	ESR
-SH	-	0.99**	0.036	**-0.504****	-0.148	-0.048
-SH+-SS	0.99**	-	0.170	**-0.532****	-0.185	-0.075
-SS	0.036	0.170	-	**-0.249***	-0.211	-0.186
IMA	**-0.504****	**-0.532****	**-0.249***	-	**0.238***	0.017
CRP	-0.148	-0.185	-0.211	**0.238***	-	0.071
ESR	-0.048	-0.075	-0.186	0.017	0.071	-

*All values were calculated using the Pearson correlation test. *p<0.05, **p<0.01. -SS: Disulphide, -SH+-SS: Total thiol, -SH: Native thiol, IMA: Ischemia modified albumin, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate*

It has been shown that there an increase in IMA levels in diseases accompanied by vascular endothelial dysfunction. In our study, IMA plasma levels were found to be significantly higher in Behçet's patients (0.96±0.06 ABSU) compared to the control group (0.90±0.06 ABSU) (p<0.001). Similarly, previous studies have shown that the MA level is higher in BD than in healthy individuals. In a study by Eryavuz Onmaz et al.[28] between Behçet's and the control group with 35 individuals in each group, IMA plasma levels were found to be significantly higher in Behçet's patients (0.63±0.11 ABSU) compared to the control group (0.51±0.15 ABSU) (p<0.001). In another study by Keskin et al.[29] on 45 healthy individuals and 57 Behçet's patients, IMA, total oxidant levels, total antioxidant levels (TAS), oxidative stress index (OSI), ESR and CRP levels were found to be significantly higher in BD, but it has been reported that only IMA among these markers can be a useful biomarker in distinguishing the active and inactive phase of the disease (p<0.01). In another study conducted with 26 Behçet's patients and 28 controls, IMA plasma levels were found to be statistically significantly higher in patients with active BD (0.93±0.13 ABSU) compared to individuals with inactive disease (0.82±0.14 ABSU) and control group (0.83±0.07 ABSU) (p<0.05). [30] In a study by Capkin et al.[31] consisting of 35 Behçet's patients and 31 healthy control groups, it was shown that IMA levels were significantly higher in the Behçet group (0.63±0.25 ABSU) compared to individuals in the control group (0.42±0.12 ABSU) (p<0.001). In this study, IMA was also found to be significantly higher in patients with BD (n=11) than in patients without vascular involvement (n=23) (0.78±0.37 and 0.56±0.14, respectively) (p<0.05). In our study, although IMA levels were found to be higher in BD compared to controls, they were found to be similar between the active and inactive disease stages.

Thiol groups are sulfurous protein molecules with antioxidant properties, which are found in human serum due to amino acids and albumin. Thiols can enter an oxidation reaction with reactive oxygen radicals and form disulfide bonds.[32] Disulfide bonds are covalent bonds and exist as oxidized forms. The disulfide bonds formed can be reduced back to thiol groups by a reversible reaction. The balance formed by this cycle is required for the antioxidant defense system and apoptosis control. Dysfunctions occurring in this cycle may increase reactive oxygen radicals, resulting in endothelial damage and the development of apoptosis.[33,34] In a study by Kose et al.[35], it was reported that plasma thiol levels were decreased in Behçet's patients (n=24) compared to healthy controls (n=30) (p<0.001) and that plasma antioxidant defense systems in BD might be insufficient or impaired due to the decrease in thiol levels. In another study consisting of 150 Behçet's patients and 100 healthy controls, serum -SH+-SS, -SH levels, and -SH/(-SH+-SS) ratio was found to be significantly lower in the Behçet patient group compared to the control group (p<0.001). Additionally, in this study, -SS/-SH and -SS/(-SH+SS) rates were found to be significantly higher in Behçet's patients compared to controls (p<0.001).[36] In a study by Balbaba et al.[37] in active ocular Behçet (n=20), inactive ocular Behçet's (n=20) and healthy control groups (n=20), -SH+-SS, -SH levels and -SH/(-SH+-SS) ratio were found to be significantly lower in ocular Behçet's patients compared to controls (p<0.001), whereas -SS/-SH ratio and -SS/(-SH+-SS) ratio was found to be significantly higher in Behçet's patients compared to controls (p<0.001). Similar to these studies, in our study, serum -SH+-SS and -SH levels (p<0.001) and -SH/(-SH+-SS) ratio (p<0.01) was lower in the Behçet group compared to the controls, and -SS/-SH and -SS/(-SH+-SS) ratios (p<0.01) were found to be significantly higher. In our study, no difference was found in terms of thiol groups between those with and without mucocutaneous, neurological, vascular, or ocular involvement in BD. Additionally, -SH/IMA and (-SH+-SS)/IMA ratios, which have not been evaluated before, were found to be significantly lower in Behçet's patients compared to controls (p<0.001).

Conclusion

The absence of specific laboratory findings in the diagnosis of BD causes inconsistencies in the diagnosis of the disease. Although some acute phase reactants such as CRP and ESR increase in BD, these markers are not specific

to the disease. Therefore, there is a need for more specific biomarkers that can be used in the diagnosis of the disease. According to the data we obtained from our study, we found that serum levels of IMA in BD are high, and the ratios of -SS/-SH and -SS/(-SS+-SH) are significantly low. The most important feature of our study is that it is the first study to evaluate IMA and thiol levels together in BD. The most important limitation of our study is that it is a cross-sectional study and only few patients were included in the study.

Ethics

Ethics Committee Approval: The research protocol was approved by the Ankara Yıldırım Beyazıt University Faculty of Medicine Research Ethics Committee (approval number: 1613, date: 14.04.2021).

Informed Consent: All patients gave informed written consent to participation in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.K., Design: A.K., Data Collection or Processing: A.K., Y.M., E.A., K.G., Analysis or Interpretation: A.K., E.F.O., Ö.E., Literature Search: A.K., Writing: A.K.

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