*Türk Klinik Biyokimya Derg 2022; 20(2): 73-82*
*https://doi.org/10.56615/tkbd.2022.09*
*Araştırma*

# Elevated Homocitrulline Levels in Patients with Behçet's Disease

## *Behçet Hastalarında Yükselmiş Serum Homositrüllin Düzeyleri*

**Raziye Topkafa**[*] **Abdullah Sivrikaya**[*] **Duygu Eryavuz Onmaz**[*]
**Gulsum Abuşoğlu**[**] **Muhammet Limon**[***] **Sema Yılmaz**[***] **Ali Ünlü**[*]

[*] Department of Biochemistry, Selcuk University Faculty of Medicine, Konya, Turkey
[**] Department of Medical Laboratory Techniques, Selcuk University Vocational School of Health, Konya, Turkey
[***] Department of Rheumatology, Selcuk University Faculty of Medicine, Konya, Turkey

**Başvuru Tarihi:** 21 Şubat 2022 **Kabul Tarihi:** 16 Haziran 2022

**ABSTRACT**

**Aim:** Homocitrulline (Hcit) is a modified amino acid originated by carbamylation of lysine residues and it is considered a potential biomarker in inflammatory diseases. Behçet's disease (BD) is an autoinflammatory disease, the diagnosis of the disease is based on clinical findings. Our aim was to investigate Hcit levels in inactive, active patients with BD and healthy subjects and to contribute to the elucidation of the relationship between Hcit levels and pathogenesis and activation of BD.

**Material and Methods:** The study included 26 active, 34 inactive patients with BD, and 30 healthy volunteers. Serum Hcit and lysine levels were quantified using the tandem mass spectrometric method (LC-MS/MS). Serum high sensitivity-CRP (hs-CRP) levels were quantified by IMMAGE 800 system (Beckman Coulter, Brea, USA). Various hematological and biochemical parameters of patients were quantified by Beckman Coulter LH 780 and Beckman-Coulter AU 5800, respectively.

**Results:** Hcit/lysine ratio (μmol Hcit/mol lysine) was statistically significantly higher in patients with BD [844.6(453.8-5975.4)] than healthy controls [334.9(117-509.3), ($p<0.002$)] and was remarkably higher in the active group [1307.0(453.8-5975.4)] compared to the inactive group [666.5(462.6-1777.2), $p=0.005$]. Moreover, Hcit/lysine ratios were positively correlate with hs-CRP ($r= 0.353$, $p=<0.001$).

**Conclusion:** Our findings showed that serum Hcit levels were high in patients with BD, and serum Hcit levels were correlated with the inflammatory load. Increased Hcit concentrations may be related to the autoinflammatory process and vascular damage in BD.

***Key words:*** *Behçet's disease; homocitrulline; inflammation; vascular complication.*

Raziye Topkafa : https://orcid.org/0000-0002-4004-5487
Abdullah Sivrikaya : https://orcid.org/0000-0003-2956-5681
D. Eryavuz Onmaz : https://orcid.org/0000-0001-8564-1824
Gülsüm Abuşoğlu : https://orcid.org/0000-0003-1630-1257
Muhammet Limon : https://orcid.org/0000-0002-5693-7885
Sema Yılmaz : https://orcid.org/0000-0001-5076-1500
Ali Ünlü : https://orcid.org/0000-0002-9991-3939
Etik onay : Selçuk Üniversitesi Tıp Fakültesi, Etik Kurulu'ndan 03 Ocak 2018 tarihi ve 2018/08 sayılı kurul kararı

**Yazışma adresi:** Duygu Eryavuz Onmaz
Selçuk Üniversitesi Tıp Fakültesi
Biyokimya ABD
Alaaddin Keykubat Kampüsü,
42075 Selçuklu, Konya
E-mail: duygu_eryavuz@hotmail.com

## ÖZET

**Amaç:** Homositrulin (Hcit), lizin kalıntılarının karbamilasyonundan kaynaklanan modifiye bir amino asittir ve inflamatuar hastalıklarda potansiyel bir biyobelirteç olabileceği düşünülmektedir. Behçet hastalığı (BH) otoinflamatuar bir hastalıktır, hastalığın tanısı klinik bulgulara dayanır. Amacımız aktif, inaktif BH olan bireylerde ve sağlıklı kişilerde Hcit düzeylerini araştırmak ve Hcit düzeyleri ile BH'nin patogenezi ve aktivasyonu arasındaki ilişkinin aydınlatılmasına katkıda bulunmaktı.

**Gereç ve Yöntem:** Çalışmaya 26 aktif, 34 inaktif BH tanısı alan birey ve 30 sağlıklı gönüllü dahil edildi. Serum Hcit ve lizin seviyeleri, tandem kütle spektrometrik yöntem (LC-MS/MS) ile ölçüldü. Serum yüksek hassasiyetli-CRP (hs-CRP) seviyeleri IMMAGE 800 sistemi (Beckman Coulter, Brea, ABD) ile ölçüldü. Hastaların çeşitli hematolojik ve biyokimyasal parametreleri sırasıyla Beckman Coulter LH 780 ve Beckman-Coulter AU 5800 cihazları ile ölçüldü.

**Bulgular:** Hcit/lizin oranı (μmol Hcit/mol lizin) Behçet hastalarında [844.6(453.8-5975.4)] sağlıklı kontrollerden [334.9(117-509.3), ($p<0.002$)] istatistiksel olarak anlamlı düzeyde yüksekti ve aktif grupta [1307.0(453.8-5975.4)], inaktif gruba göre [666.5(462.6-1777.2), $p=0.005$] istatiksel olarak anlamlı düzeyde yüksekti. Ayrıca, Hcit/lizin oranları, hs-CRP düzeyleri ile pozitif koreleydi ($r= 0.353$, $p=<0.001$).

**Sonuç:** Bulgularımız, Behçet hastalarında serum Hcit düzeylerinin yüksek olduğunu ve serum Hcit düzeylerinin inflamatuar yük ile korele olduğunu gösterdi. Artmış Hcit konsantrasyonları, BH'de otoinflamatuar süreç ve vasküler hasar ile ilişkili olabilir.


**Anahtar kelimeler:** Behçet hastalığı; homositrülin; enflamasyon; vasküler komplikasyon.

## INTRODUCTION

Behçet's disease (BD) is an autoimmune, inflammatory, chronic disease with oral and genital aphthous ulcers, skin lesions, and uveitis. BD was first defined by Dr. Hulusi Behçet in 1937 (1). BD is a multisystemic disease that may affect the gastrointestinal, cardiovascular, pulmonary, musculoskeletal, and central nervous systems. Although the BD has a worldwide distribution, it is more common, especially along the Silk Road, a historic trade route between East Asia and the Mediterranean (2). BD is most commonly seen in Turkey, with a prevalence of 421/10000; Iran, Israel, and Japan follow it. The mean diagnosis age of the BD is 20-40 years and is rare to be observed in children and individuals over 50 years of age (3). The etiology and pathogenesis of BD are still unclear. However, it is considered that autoimmune and inflammatory disorders, genetic predisposition, and environmental factors may play a vital role in the pathology of BD (4). HLA-B51 antigen is shown as the most critical genetic predisposition factor in BD (5). BD frequency is associated with up to 80% HLA-B51 antigen in Asian countries on the Silk Road and up to 55% in Japan (6). Environmental factors, including mechanical elements, smoking patterns, and food consumption, especially infectious agents, are essential in the pathogenesis of BD. Although the clinical manifestations of BD are variable, the most common are skin-mucosal lesions (7). Because there is not a single specific marker or specific laboratory test for disease diagnosis, the diagnosis is made with the criteria recommended by the International Study Group (8). However, some studies have shown that various biochemical and hematologic parameters such as neutrophils (NEU), white blood cells (WBC), and platelets (PLT) are elevated in patients with BD (9). Inanır et al. reported that erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were statistically significantly higher ($p <0.05$) in the BD group than the control group (10). Aksoy et al. reported that ESR, high-sensitivity C-reactive protein (hs-CRP), and red cell distribution width (RDW) levels in BD were higher than the healthy subjects (11). Briefly, various studies have reported different routine parameters such as ESR, CRP, and RDW increase in BD, but the changes in these parameters are not always correlated with clinical activity. Therefore, various studies have been conducted on different candidate markers for diagnosing the disease. Karatay et al. showed that vitamin D levels were reduced in patients with BD (12). Mesci et al. reported that serum pentraxin-3 levels were significantly higher in BD compared to the control group (13). Sarican et al. reported that homocysteine concentrations were significantly higher in active BD than inactive BD and healthy subjects (14). Studies investigating potential

markers that may be useful in diagnosing BD are increasing.

Carbamylation is a non-enzymatic post-translational modification that occurs by binding isocyanic acid to amino groups of proteins (15). The most well-known carbamylation product is homocitrulline (Hcit) formed by converting lysine residues of proteins (16). Recent studies have indicated that Hcit is a candidate biomarker for monitoring various diseases such as chronic renal failure, cardiovascular diseases, and rheumatoid arthritis (17-19). Increasing evidence suggests that protein carbamylation is a significant risk factor, especially for atherogenesis and endothelial dysfunction. Vasculitis and perivascular infiltration that affects vessels of various sizes is one of the main pathological processes in BD. It has also been shown that carbamylated proteins induce the inflammatory response and are involved in autoimmune diseases' pathogenesis (20). Therefore, considering the vascular and inflammatory pathology of BD, the study aimed to investigate serum Hcit as a possible marker in the diagnosis of BD. However, various difficulties were encountered in measuring Hcit levels. Initially, colorimetric and ninhydrin-based chromatographic methods were developed to measure Hcit levels, but the desired sensitivity and reliability were not achieved in these methods (21). With LC-MS / MS-based methods, Hcit levels could be measured as sensitive, specific, and reliable (22). We aimed to investigate Hcit levels in inactive, active patients with BD and healthy subjects and contribute to the elucidation of the relationship between Hcit levels and pathogenesis and activation of BD.

## MATERIALS and METHODS

### *Study design*

### *Patients*

The study included 60 patients diagnosed with BD according to the criteria determined by the International Study Group and admitted to the Selcuk University Faculty of Medicine Rheumatology outpatient clinic. 26 patients were included in the active group if they had at least two clinical findings: oral aphthae, genital ulceration, skin findings, joint, eye, neurological, and gastrointestinal system involvement, while 34 patients without active findings were included in the inactive group by clinicians. 30 healthy volunteers with similar age distribution were also included in the study. Subjects with chronic kidney, liver disease, diabetes, cardiovascular disease and risk factors, other rheumatic diseases, neurodegenerative disease, and infectious disease were excluded from the study. Venous blood samples of all participants were taken to serum separator tubes and tubes containing K2-EDTA after 12-h overnight fasting. Separated serum samples were stored at -80 °C by analysis time. All patients provided written informed consent. This study was approved by Selcuk University Faculty of Medicine ethics committee (Number: 2018/08, Date:03/01/2018).

### *Laboratory analysis*

Serum creatinine (CRE) and hs-CRP levels were measured with Beckman-Coulter AU 5800 (Beckman Coulter, Brea, USA) and IMMAGE 800 system (Beckman Coulter, Brea, USA), respectively. Total white blood cell count (WBC), lymphocyte (LYM), neutrophil (NEU), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), platelet (PLT) count, red blood cell count (RBC), mean platelet volume (MPV), platelet distribution width (PDW), hematocrit (HCT) levels were measured using complete blood samples by Beckman Coulter LH 780 analyzer (Beckman Coulter, Miami, FL, USA). The erythrocyte sedimentation rate (ESR) was measured with Alifax (Padova, Italy).

### *Serum Hcit analysis*

Hcit and lysine levels were quantified by modifying the method developed by Dietzen et al (23). Briefly, 200 μl of each standard and samples were taken into Eppendorf tubes, and 50 μl of d4-L-citrulline and 50 μl of d8-L-lysine were added to internal standards. To

precipitate proteins, 850 µl of methanol was added to the mixture, and it was vortexed for 30 seconds, then incubated for 10 minutes at room temperature. They were centrifuged at 13000 rpm for 5 minutes, and the supernatant was taken into reaction tubes and then evaporated under nitrogen gas at 65 °C. 200 µl of 3N HCl / n-butanol mixture was added after evaporation was completed. The tubes were sealed and kept at 65 °C for 30 minutes. After incubation, the evaporation process was repeated. The residues were dissolved in 250 µL of acetonitrile: water, including 0.1% formic acid mixture (20:80; v:v%), and 40 µL was injected into LC-MS/MS system.

The analytes were detected with API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) equipped with an electrospray ionization interface.

Separation of analytes was performed using a Shimadzu HPLC system (Kyoto, Japan) and Phenomenex C18 HPLC column (50 mm x 4.6 mm, part no: 00B-4041-E0). The mobile phase consisted of 0.1% formic acid and acetonitrile mixture (80:20; v: v%). Total flow rate, run time, and column oven temperature were 1 mL/min, 5 min, and 40 °C, respectively.

The Q1 to Q3 ion transitions were 246.0/127.0, 203.0/84.0, 236.0/74.0, and 203.0/84.0 for Hcit, lysine, d4-L-citrulline, and d8-L-lysine, respectively. Method optimization parameters including ion spray voltage, temperature, ion source (GS1) and ion source (GS2), curtain, collision gas values were adjusted as 5000 V, 400 $^{O}$C, 40, 60, 20, and 5 psi, respectively. The intra- and inter-assay imprecision values of the method for Hcit are 4.3% and 6.4%, respectively. The recovery value is 97.8%.

### *Statistical analysis*

SPSS statistical software package version 21.0 was used for statistical analysis. The distribution of data was determined with the One-Sample Kolmogorov-Smirnov test. Student's T-test and Mann-Whitney U tests were performed to compare the mean and median values of the two groups, respectively. One-way ANOVA analysis (post-hoc analysis with Tukey or Tamhane's T2 tests) and Kruskal – Wallis test (post-hoc analysis Mann-Whitney U) were respectively performed for comparing mean and median values between more than two groups. The correlation analysis was performed with Spearman and Pearson correlation tests. $P<0.05$ was accepted as statistically significant. Medcalc software package version 19.4 was used for Receiver Operating Characteristic (ROC) curve analysis.

## RESULTS

The study included 26 active (18 male and 8 female), 34 inactive (23 male and 11 female) patients with Behçet, 30 healthy subjects (20 male and 10 female). The mean age of inactive, active Behçet, and control groups were 44.2±12.5, 44.4±15.6 and 44.7±2.3 years, respectively. The mean ages of the three groups were similar ($p=0.985$). Clinical findings of patients were shown in Table 1.

**Table 1.** Clinical manifestations of the patients with Behçet's Disease.
**Tablo 1.** Behçet hastalarının klinik belirtileri.

| | Number | Percent |
|---|---|---|
| Oral aphthae | 50 | 83.3 |
| Genital ulceration | 37 | 61.7 |
| Vascular involvement | 35 | 58.3 |
| Neurological involvement | 10 | 16.7 |
| Ocular lesion | 32 | 53.3 |
| Skin involvement | 31 | 51.7 |
| Pathergy positive | 40 | 76.7 |

Serum Hcit concentration was found to be statistically significantly higher in the Behçet group [0.87(0.43-13.92) mol] than in the healthy subjects [0.38(0.16-0.61) mol, $p<0.001$], while lysine levels were statistically significantly lower in the Behçet group [989.7(369.2-3273.9) mol, $p=0.048$]. Hcit/lysine ratio (µmol Hcit/mol lysine) was statistically significantly higher in Behçet group [844.6(453.8-5975.4) µmol Hcit/mol lysine] than in healthy controls [334.9(117-509.3) µmol Hcit/mol lysine, $p<0.001$]. Serum Hcit concentration was found to be statistically significantly higher in the active subgroup [1.28(0.43-13.92) mol] compared to the inactive subgroup [0.76(0.45-3.26)

mol, p=0.001]. In contrast, no significant difference was found between serum lysine levels (p=0.296). Hcit/lysine ratio was statistically significantly higher in the active group [1307.0(453.8-5975.4) μmol Hcit/mol lysine compared to the inactive group [666.5(462.6-1777.2) μmol Hcit/mol lysine, p=0.005]. Compared to the inactive subgroup control group, Hcit concentration (p<0.001) and Hcit/lysine ratio (p<0.001) were found higher in the inactive group, while no significant difference was found between lysine levels (p=0.282). The comparison of serum Hcit, lysine, and Hcit/lysine ratio in patients with BD and healthy subjects was shown in Table 2.

Moreover, Hcit/lysine ratios were positively correlate with CRP (r= 0.353, p=<0.001), PLR (r= 0.348, p=0.001), PLT (r= 0.345, p=0.001), urea (r= 0.342, p=0.002) NLR (r= 0.248, p=0.018), RDW (r= 0.263, p=0.012), ESR (r= 0.262, p=0.013), NEU (r= 0.208, p=0.049) and negatively correlate with eGFR (r=-0.414, p<0.001). The level of laboratory parameters of the patient and control groups were shown in Table 3.

Area under curve (AUC) values were determined as 0.997 (95% CI 0.923 to 0.988, p <0,0001), 0.955 (95% CI 0.914 to 0.984, p <0,0001), 0.777 (95% CI 0.669 to 0.886, p=0.001), 0.737 (95% CI 0.623 to 0.850, p=0.001), 0.734 (95% CI 0.615 to 0.854, p=0.005), 0.726 (95% CI 0.598 to 0.855, p=0.001), 0.723 (95% CI 0.604 to 0.841, p=0.007), 0.701 (95% CI 0.576 to 0.827, p=0.015), 0.637 (95% CI 0.508 to 0.766, p=0.049) for serum Hcit/lysine ratio, Hcit, RDW, hs-CRP, NLR, NEU, PLR, PLT, ESR respectively. The sensitivity and specifity were found to be 98% and 96.7% for 442.15 μmol Hcit/mol lysine cut-off value. Reciever operating curves (ROCs) for serum Hcit/lysine ratio, hs-CRP, RDW, NEU, NLR, PLR were shown in Figure 1.

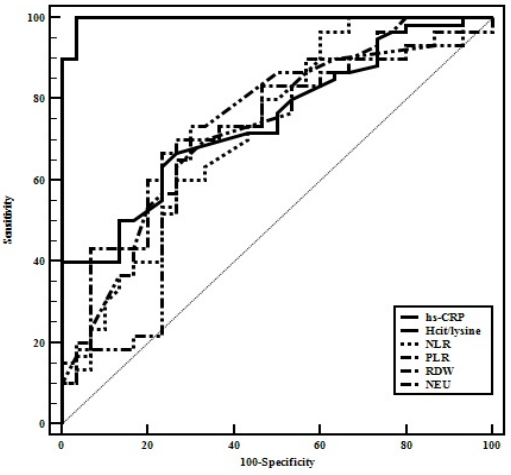


**Figure 1.** Reciever operating curves (ROCs) for serum Hcit/lysine ratio, hs-CRP, RDW, NEU, NLR, PLR.

**Table 2.** Comparison of serum Hcit, lysine and Hcit/lysine ratio in patients with BD and healthy subjects.
**Tablo 2.** Behçetli hastalarda ve sağlıklı deneklerde serum Hcit, lisin ve Hcit/lizin oranının karşılaştırılması.

| | Behçet group | Control group | Inactive group | Active group | p |
|---|---|---|---|---|---|
| Hcit (mol) | 0.87 (0.43-13.92) | 0.38 (0.16-0.61) | 0.76 (0.45-3.26) | 1.28 (0.43-13.92) | **a:p<0.001** **b:0.001** **c:p<0.001** **d:p<0.001** |
| Lysine (mol) | 989.7 (369.2-3273.9) | 1212.3 (876.7-2075.3) | 1082.19 (369.2-3273.9) | 856.16 (517.8-2739.7) | **a:0.048** b:0.296 **c:0.013** d:0.282 |
| μmol Hcit/mol lysine | 844.6(453.8-5975.4) | 334.9 (117-509.3) | 666.5 (462.6-1777.2) | 1307.0 (453.8-5975.4) | **a:p<0.001** **b:0.005** **c:p<0.001** **d:p<0.001** |

p <0.05 value was accepted as the significance value, a: Behçet group vs control group, b: active subgroup vs inactive subgroup, c: active subgroup vs control group, d: inactive subgroup vs control group.

**Table 3.** Laboratory parameters of patients with BD and healthy controls.
**Tablo 3.** BB'li hastalar ve sağlıklı kontrollerin laboratuvar parametreleri.

| | Behçet group | Control group | Active group | Inactive group | p |
|---|---|---|---|---|---|
| WBC (k/μL) | 7.40±2.01 | 7.29±1.91 | 8.15±2.45 | 6.83±1.38 | a:0.812<br>**b:0.030**<br>c:0.304<br>d:.0.617 |
| PLT (k/μL) | 295.9±49.7 | 253.2±58.9 | 317.8±55.9 | 279.2±37.1 | **a:0.001**<br>**b:0.011**<br>**c:p<0.001**<br>d: 0.105 |
| MCV (fl) | 86.95±7.38 | 87.10±3.99 | 84.3±3.6 | 88.9±8.7 | a:0.919<br>**b:0.020**<br>**c:0.022**<br>d:0.597 |
| RBC ($10^6$/μL) | 4.90±0.58 | 4.80±0.44 | 5.05±0.34 | 4.78±0.68 | a:0.396<br>b:0.140<br>c:0.053<br>d:0.993 |
| LYM (k/μL) | 2.21±0.85 | 2.34±0.89 | 2.45±1.02 | 2.03±0.67 | a:0.521<br>b:0.202<br>c:0.967<br>d:0.471 |
| MCHC (g/dL) | 32.83±0.97 | 33.47±0.83 | 32.82±1.1 | 32.81±0.87 | **a:0.002**<br>b:0.932<br>**c:0.038**<br>**d:0.018** |
| ESR (mm/hr) | 18.5±7.83 | 10.3±3.31 | 22.2±9.1 | 13.1±5.2 | **a:0.013**<br>**b:0.006**<br>**c:p<0.001**<br>d:0.343 |
| HGB (g/dL) | 14.0(1.19-17.0) | 14.1(12-18) | 14.0(1.2-16.20) | 14.0(9.10-17.0) | a:0.691<br>b:0.811<br>c:0.767<br>d:0.706 |
| MCH (pg) | 28.0(21.9-34.4) | 29.4(26.0-40.0) | 27.5(23.3-31.0) | 30.2(21.9-34.4) | a:0.240<br>**b:0.014**<br>**c:0.003**<br>d:0.562 |
| RDW (%) | 15.0(11.4-19.5) | 14.0(12.0-18.0) | 15.0(13.0-18.0) | 15.0(11.4-19.5) | **a:0.001**<br>b:0.511<br>**c:0.001**<br>**d:0.013** |
| PDW (%) | 16.6(9.5-18.9) | 16.5(15.0-18.0) | 16.3(14.7-18.9) | 17.0(9.5-18.3) | a:0.980<br>b:0.119<br>c:0.339<br>d:0.457 |
| MPV (fl) | 8.1(6.9-11.0) | 8.3(6.7-15.0) | 7.9(6.9-11.0) | 8.3(7.1-9.9) | a:0.201<br>b:0.570<br>c:0.143<br>d:0.404 |
| NEU (k/μL) | 4.8(3.2-9.5) | 3.9(1.3-6.2) | 5.0(3.4-9.5) | 4.6(3.2-7.1) | **a:0.001**<br>b:0.141<br>**c:0.002**<br>**d:0.012** |
| NLR | 2.4(1.3-5.9) | 1.7(0.5-5.2) | 2.59(1.33-5.5) | 2.30(1.55-5.94) | **a:0.001**<br>b:0.765<br>**c:0.005**<br>**d:0.002** |
| PLR | 156.3(66.1-317.4) | 109.3(40-264.6) | 156.1(66.1-278.9) | 156.5(76.8-317.4) | **a:p<0.001**<br>b:0.612<br>**c:0.001**<br>**d:0.003** |
| CRE (mg/dL) | 0.72(0.42-1.13) | 0.70(0.55-1.19) | 0.73(0.42-0.85) | 0.71(0.59-1.13) | a:0.672<br>b:0.680<br>c:0.564<br>d:0.252 |
| eGFR (mL/min) | 120.0±11.71 | 127.01±22.74 | 117.4±11.6 | 122.2±11.0 | a:0.594<br>b:0.149<br>c:0.058<br>d:0.694 |
| hs-CRP (mg/L) | 3.06(0.36-13) | 1.75(0.32-4.0) | 4.75(0.36-12) | 2.19(0.44-13) | **a:p<0.001**<br>**b:0.029**<br>**c: p<0.001**<br>**d:0.011** |

p <0.05 value was accepted as the significance value, a: Behçet group vs control group, b: active subgroup vs inactive subgroup, c: active subgroup vs control group, d: inactive subgroup vs control group.

## DISCUSSION

BD is a multisystemic, recurrent, chronic, and inflammatory disorder. The laboratory findings of the disease are not specific, and the diagnosis is generally based on the clinical evaluation of the subjects. Therefore, new markers are needed, especially in the diagnosis and treatment process of disease (1,2).

Carbamylation is a non-enzymatic modification formed by the binding of isocyanic acid to the amino group of proteins. This reaction leads to changes in structural and functional characteristics of proteins, leading to their molecular aging. Carbamylated proteins exhibit harmful effects in biological systems and are related to the pathogenesis of various inflammatory and chronic diseases such as atherosclerosis, rheumatoid arthritis, and chronic kidney disease. Therefore, carbamylated proteins may be valuable biomarkers for many diseases, and research on carbamylated proteins has increased considerably lately. Hcit is the most characteristic carbamylation product, and it can be measured as sensitive and specific by mass spectrometry-based methods.

In the study performed by Jaisson et al. it was found that altered Hcit concentrations at the beginning, sixth and twenty-fourth months of treatment of hemodialysis patients with chronic renal failure. A total of 108 patients were evaluated for HCit levels, and the value of 1000 μmol / mol lysine before treatment decreased by 50% in the sixth month of treatment and remained constant over the twenty-four-month period. Therefore, it was emphasized that Hcit is a reliable biomarker in the follow-up of chronic renal failure patients (24).

A study investigating the relationship between serum Hcit and coronary artery disease; Hcit levels of 45 patients and 109 controls. It was demonstrated Hcit levels (0.17–0.25 mmol/mol lysine) were higher in the patient group than in the control group (0.10–0.15 mmol/mol lysine) (25) .

Desmons et al. performed a study that included 145 patients with chronic renal failure, 29 patients with chronic renal failure complicated with acute renal failure, 39 patients with acute renal failure. Serum Hcit concentrations found in the group with chronic renal failure were significantly higher ($p<0.05$) than in the group with acute renal failure in this study (26).

Although citrulline and Hcit are post-translational modification products (citrullination and lysine carbamylation, respectively), they cause similar effects on proteins, altering proteins immunogenicity and inducing autoantibody response. In the sera of patients with rheumatoid arthritis, antibodies specific to citrullinated and homocitrullinated proteins were detected. Antibodies binding to citrullinated proteins (ACPA) is a finding specific to rheumatoid arthritis. The ACPA antigens described so far are naturally occurring proteins in the human body. Citrullinated fibrin, vimentin, and histones are considered possible autoantigens for ACPAs in rheumatoid arthritis. Carbamylation can change the charge of proteins and prevent their biological functions, similar to citrullination. Carbamylation increases the reactivity of proteins to citrullinated peptides by causing an increase in their immunogenicity. Homocitrullinated proteins were also found in the joints of patients with rheumatoid arthritis. IgG and IgA anti-carbamylated protein (anti-CarP) antibodies were demonstrated in approximately 45% of patients with rheumatoid arthritis against carbamylated proteins. Anti-carP antibodies do not cross-react with ACPA, and IgG anti-CarP antibodies have been shown in 16% of ACPA-negative patients. Anti-CarP antibodies are considered new candidate marker antibodies in the diagnosis of rheumatoid arthritis (19).

In summary, carbamylated proteins are involved in the pathogenesis of autoinflammatory diseases by triggering the immunogenic and inflammatory response, so carbamylated proteins and autoantibodies against them are considered potential markers in diagnosing inflammatory diseases. Considering the autoinflammatory nature of BD, Hcit may be a candidate marker for BD and activation of disease.

Furthermore, vascular involvement is one of the severe involvement of BD. It can affect almost all of the vessels in the body. However, vein involvement is more common than arterial involvement (27). Carbamylation involves the pathogenesis of atherosclerosis, thrombus formation, and coronary artery disease. Protein carbamylation has been closely linked to the risk of cardiovascular diseases. Carbamylated proteins promote endothelial dysfunction. For example, carbamylated LDLs play an essential role in atherosclerosis with enhanced susceptibility to oxidation and adhesion to monocytes in endothelial cells. Moreover, it can function as the causative agent of thromboembolic events in other carbamylated proteins localized in the extracellular matrix (28).

Various biomarkers have been evaluated to assess carbamylation; Hcit and albumin are frequently used. Most of the potential markers are not clinically useful due to difficulties in measuring (29). Hcit is considered a reliable worldwide marker of protein carbamylation. Hcit is a metabolite produced due to the reaction of lysine with cyanate. Cyanate is produced with various pathways, such as urea deamination in the body or by oxidation of thiocyanate through the myeloperoxidase (MPO) enzyme. Cyanate is present in equilibrium with urea in physiological conditions. Increasing urea concentration in renal failure causes carbamylation of many proteins. Apart from urea, the other way of forming cyanate is carried out by MPO released from inflammation areas, including atherosclerotic plaques and environmental factors. MPO is a heme protein abundant in neutrophils, monocytes, and specific tissue macrophages, such as in human atheroma. MPO is a potential participant in many stages of the atherosclerotic process, so MPO catalyzed protein carbamylation also increases in chronic inflammatory diseases such as atherosclerosis, where MPO is catalytically active. Studies in transgenic mMPO-knockout mice expressing human MPO have shown that atherosclerosis accelerates, and Hcit concentrations increase in aortic tissue, correlated with aortic cholesterol content (30). In an age and gender-matched case-control study with 150 patients with cardiovascular disease and 300 controls, a correlation was found between the prevalence of coronary artery disease, peripheral artery disease, and Hcit levels. It has been demonstrated that individuals with Hcit concentration ≥300 μmol / mol lysine have 7-8 times more clinical and angiographic cardiovascular disease and adverse cardiac event risk than those with ≤30 μmol Hcit / mol lysine. In another age and gender-matched case-control study, increased plasma Hcit levels could be an independent predictor of the risk of cardiovascular diseases, myocardial infarction, stroke, and death (20). Considering venous pathology, thrombotic complications, cardiac involvement, and the autoinflammatory nature of BD, Hcit can be a risk factor in BD pathogenesis and activation.

Our study evaluated whether Hcit could be a marker for BD by measuring Hcit levels in 30 healthy subjects and 60 patients with BD without any renal disorder or other inflammatory diseases. It was demonstrated that Hcit/lysine ratio was significantly higher in the Behçet group than in healthy controls ($p<0.001$), while lysine levels were statistically significantly lower in the Behçet group ($p=0.048$). Hcit/lysine ratio (μmol Hcit/mol lysine) was statistically significantly higher in Behçet group than in healthy controls ($p<0.001$). Serum Hcit concentration was statistically significantly higher in the active subgroup than in the inactive subgroup ($p=0.001$), while no significant difference was found between serum lysine levels ($p=0.296$). Hcit/lysine ratio was statistically significantly higher in the active group compared to the inactive group. Compared to the inactive subgroup control group, Hcit concentration ($p<0.001$) and Hcit/lysine ratio ($p<0.001$) were found higher in the inactive group, while no significant difference was found between lysine levels ($p=0.282$). Our findings support that Hcit may be associated with BD activation and pathogenesis. Hcit levels may be significantly increased in BD and the active group related to vascular complications

in BD or triggering the inflammatory response due to an increase in the immunogenicity of proteins.

In conclusion, our study showed that Hcit levels increased in patients with BD and correlated with disease activity. Our study is essential for being the first study to measure Hcit levels in patients. Increased Hcit concentrations may be related to vascular damage and the autoinflammatory process in BD, thereby being a significant risk factor for BD activation and pathogenesis. However, the inclusion of a limited number of patients and healthy controls, MPO and citrulline levels not measured is the lack of our study.

**ACKNOWLEDGEMENTS**

The authors thank the Selcuk University for funding this Project. This study was supported by Selcuk University Scientific Research Projects Coordinator (grant number 18202006).

**COMPETING INTERESTS**

Authors have no conflict of interests.

**FINANCIAL DISCLOSURE:** This study was supported by Selcuk University Scientific Research Projects Coordinator (grant number 18202006).

**REFERENCES**

1. Gazioglu S, Boz M, Arica D, Boz C. Nöro-Behçet Hastalığında Klinik Özellikler. Dicle Tıp Dergisi. 2020;47:324-30.
2. Woźniacka A, Sysa-Jędrzejowska A, Jurowski P, Jablkowski M, Kot M. Morbus Behçet – a rare disease in Central Europe. Arch Med Sci. 2015; 11(6):1189–1196.
3. Hiyama T, Harada Y, Doi T, Kiuchi Y. Early administration of adalimumab for paediatric uveitis due to Behçet's disease. Pediatr Rheumatol. 2019;17:29.
4. Nair JR, Moots RJ. Behcet's disease. Clin Med. 2017; 17(1):71-77.
5. Scherrer MAR, Rocha VB, Garcia LC. Behçet's disease: review with emphasis on dermatological aspects. An Bras Dermatol. 2017; 92(4):452-464.
6. 6. Shenavandeh S, Jahanshahi KA, Aflaki E, Tavassoli A. Frequency of HLA-B5, HLA-B51 and HLA-B27 in patients with idiopathic uveitis and Behçet's disease: a case-control study. Reumatologia. 2018;56:67-72.
7. Yazici H, Seyahi E, Hatemi G, Yazici Y. Behçet syndrome: a contemporary view. Nat Rev Rheumatol. 2018; 14:107.
8. Rokutanda R, Kishimoto M, Okada M. Update on the diagnosis and management of Behcet's disease. Open access rheumatology : research and reviews. 2015; 7:1-8.
9. Alan S, Tuna S, Turkoglu EB. The relation of neutrophil-to-lymphocyte ratio, platelet-tolymphocyte ratio, and mean platelet volume with the presence and severity of Behcet's syndrome. Kaohsiung J Med Sci. 2015; 31(12):626-31.
10. İnanır I, Onur E, Pırıldar T, Gündüz K, Var A. IL-2R, IL-6 and IL-8 levels in Behçet's disease. Türkdem. 2010; 44:213-5.
11. Aksoy SN, Savas E, Sucu M, Kisacik B, Kul S, Zengin O. Association between red blood cell distribution width and disease activity in patients with Behcet's disease. J Int Med Res. 2015; 43(6):765-73.
12. Karatay S, Yildirim K, Karakuzu A, Kiziltunc A, Engin RI, Eren YB, et al. Vitamin D status in patients with Behcet's Disease. Clinics (Sao Paulo) 2011; 66(5):721-723.
13. Alpaslan MB, Işbilen BB, Gül SH, Gönenli G, Kavala M, Kasapoğlu GE, et al. Can Pentraxin-3 be a candidate marker in the follow-up of the patients with Behçet's Disease?. Arch Rheumatol. 2016; 32(2):91-95.
14. Sarican T, Ayabakan H, Turkmen S, Kalaslioglu V, Baran F, Yenice N. Homocysteine: an activity marker in Behcet's disease?. J Dermatol Sci. 2007; 45(2):121-6.
15. Jaisson S, Pietrement C, Gillery P. Carbamylation-derived products: bioactive compounds and potential biomarkers in chronic renal failure and atherosclerosis. Clin Chem. 2011; 57(11):1499-1505.
16. Taga Y, Tanaka K, Hamada C, Kusubata M, Ogawa-Goto K, Hattori S. Hydroxyhomocitrulline is a collagen-specific carbamylation mark that affects cross-link formation. Cell Chem Biol. 2017; 24(10):1276-1284.
17. Desmons A, Jaisson S, Pietrement C, Rieu P, Wynckel A, Gillery P. Homocitrulline: a new marker for differentiating acute from chronic renal failure. Clin Chem Lab Med. 2016; 54(1):73-9.
18. Verbrugge FH, Tang WHW, Hazen SL. Protein carbamylation and cardiovascular disease. Kidney Int. 2015; 88(3):474-478.
19. Turunen S, Huhtakangas J, Nousiainen T, Valkealahti M, Melkko J, Risteli J. Rheumatoid arthritis antigens Hcit and citrulline are generated by local myeloperoxidase and peptidyl arginine deiminases 2, 3 and 4 in rheumatoid nodule and synovial tissue. Arthritis Res Ther. 2016; 18(1):239.

20. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Hörkkö S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Medicine. 2007; 13(10):1176-84.

21. Jaisson S, Gorisse L, Pietrement C, Gillery P. Quantification of plasma homocitrulline using hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometry. Anal Bioanal Chem. 2012; 402(4):1635-41.

22. Jaisson S, Desmons A, Doue M, Gorisse L, Pietrement C, Gillery P. Measurement of homocitrulline, a carbamylation-derived product, in serum and tissues by LC-MS/MS. Curr Protoc Protein Sci. 2018; 92(1):56.

23. Dietzen DJ, Weindel AL, Carayannopoulos MO, Landt M, Normansell ET, Reimschisel TE, et al. Rapid comprehensive amino acid analysis by liquid chromatography/tandem mass spectrometry: comparison to cation exchange with post-column ninhydrin detection. Rapid Commun Mass SP. 2008; 22(22):3481-8.

24. Jaisson S, Kazes I, Desmons A, Fadel F, Oudart JB, Santos-Weiss IC, et al. Homocitrulline as marker of protein carbamylation in hemodialyzed patients. Clin Chim Acta. 2016; 460:5-10.

25. Jaisson S, Kerkeni M, Santos-Weiss IC, Addad F, Hammami M, Gillery P. Increased serum homocitrulline concentrations are associated with the severity of coronary artery disease. Clin Chem Lab Med. 2015; 53(1):103-10.

26. Desmons A, Jaisson S, Pietrement C, Rieu P, Wynckel A, Gillery P. Homocitrulline: a new marker for differentiating acute from chronic renal failure. Clin Chem Lab Med. 2016; 54(1):73-9.

27. Ataş H, Canpolat F, Eskioglu F. Evaluation of Mean Platelet Volume in Patients With Behcet's Disease as an Indicator of Vascular Thrombosis. Arch Iran Med. 2018; 21(6):234-239.

28. Jaisson S, Pietrement C, Gillery P. Carbamylation-derived products: bioactive compounds and potential biomarkers in chronic renal failure and atherosclerosis. Clin Chem. 2011; 57(11):1499-1505.

29. Delanghe S, Delanghe JR, Speeckaert R, Van BW, Speeckaert MM. Mechanisms and consequences of carbamoylation. Nat Rev Nephrol. 2017; 13(9):580-593.

30. Verbrugge FH, Tang WH, Hazen SL. Protein carbamylation and cardiovascular disease. Kidney Int. 2015; 88(3):474-478.