Original Investigation / Orijinal Araştırma

DOI: 10.4274/tod.galenos.2020.34713
Turk J Osteoporos 2020;26:104-9

Serum Melatonin Levels in Patients with Behçet's Disease

Behçet Hastalığı Olan Hastalarda Serum Melatonin Düzeyleri

Ayhan Kul, Nurinnisa Öztürk, Yaşar Arslan, Fatih Baygutalp

Atatürk University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Erzurum, Turkey

Abstract

Objective: Melatonin (MLT) hormone has been reported to play a role in the immunopathogenesis and aetiology of many chronic inflammatory diseases. We aimed to investigate the role of MLT in Behçet's disease (BD) by determining the serum MLT levels of patients with BD.

Materials and Methods: A total of 40 patients (mean age, 35.3±9.0 years; age range, 19-57 years), including 19 women and 21 men, and 40 healthy individuals, including 20 women and 20 men, matched for age and gender (mean age, 37.7±11.2 years; age range, 19-65 years) were included in this study. Serum MLT levels of the participants were determined, and their demographic data, laboratory parameters, and clinical features were recorded. Disease activity was evaluated according to the BD current activity form 2006. The relationship between disease activity and serum MLT level was examined.

Results: There were no significant differences in the demographic characteristics and other laboratory parameters between the groups, except for serum MLT levels and mean platelet volume values ($p<0.05$). Serum MLT values were found to be significantly lower in patients having headache than in those without headache ($p<0.05$), but there was no significant difference in other clinical parameters. No significant correlation was found between the serum MLT levels of patients with BD and laboratory parameters and disease activity scores.

Conclusion: Although this study provides evidence that MLT plays a possible role in the immunopathogenesis of BD in patients with a headache history, there was no association between MLT level and disease activity. We suggest that further studies are needed to determine the possible role of MLT in BD and that our findings should be evaluated by future research.

Keywords: Behçet's disease, melatonin, headache, immunopathogenesis

Öz

Amaç: Melatoninleri (MLT) hormonunun birçok kronik enflamatuvar hastalığın immünopatogenezinde ve etiyolojisinde rol oynayabileceği bildirildiğinden dolayı, çalışmamızda Behçet hastalığı (BH) olan hastaların serum MLT seviyelerinin belirlenerek hastalık aktivitesi ile arasında olası bir ilişki olup olmadığını incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmada 19 kadın ve 21 erkek olmak üzere toplam 40 hasta (ortalama yaş; 35,3±9,0 yıl; yaş aralığı; 19-57 yıl) ile yaş ve cinsiyetleri benzer olan 20 kadın ve 20 erkek toplam 40 sağlıklı kontrol (ortalama yaş; 37,7±11,2 yıl; yaş aralığı;19-65 yıl) bulunmaktaydı. Katılımcıların demografik verileri, laboratuvar ve klinik özellikleri kaydedilerek serum MLT seviyeleri belirlendi. BH anlık aktivite form-2006'ya göre hastalık aktivitesi değerlendirildi. Hastalık aktivitesi ile serum MLT seviyesi arasındaki ilişki incelendi.

Bulgular: Gruplar arasında serum MLT düzeyleri ve ortalama trombosit hacmi değerleri hariç ($p<0,05$) demografik özellikler ve diğer laboratuvar parametreleri arasında anlamlı bir fark yoktu. Hastalık aktivitesinin değerlendirilmesinde kullanılan klinik parametrelerin hastalardaki varlığına göre yapılan incelemede; baş ağrısı olanlarda olmayanlara göre serum MLT değerlerinin anlamlı şekilde daha düşük olduğu bulunurken ($p<0,05$), diğer klinik parametrelerde anlamlı bir fark yoktu. BH olan hastalarının değerlendirilen laboratuvar parametreleri ve hastalık aktivite skorları ile serum MLT seviyesi arasında anlamlı bir ilişki bulunmadı.

Sonuç: MLT'nin BH immünopatogenezinde ve baş ağrısı hikayesi olanlarda olası bir rol oynadığına dair kanıt sunmasına rağmen MLT düzeyi ile hastalık aktivitesi arasında bir ilişki bulunmadı. BH'de MLT'nin olası rolünü belirlemek için daha fazla çalışmaya ihtiyaç olduğunu ve bulgularımızın gelecekteki araştırmalarla desteklenmesi gerektiğini öneriyoruz.

Anahtar kelimeler: Behçet hastalığı, melatonin, baş ağrısı, ilişki

Address for Correspondence/Yazışma Adresi: Ayhan Kul MD, Atatürk University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Erzurum, Turkey
Phone: +90 533 576 15 14 **E-mail:** drayhankul@gmail.com **ORCID ID:** orcid.org/0000-0003-1313-9469
Received/Geliş Tarihi: 10.02.2020 **Accepted/Kabul Tarihi:** 16.04.2020

Introduction

Behçet's disease (BD) is a chronic systemic inflammatory vasculitis with unknown etiology including oral aphthous ulcers, genital ulcers, skin lesions, ocular lesions, gastrointestinal and central nervous system abnormalities and other pathologies. Vascular inflammation affects arteries and veins in all types, diameters and localization, causing endothelial cell dysfunction (1). Although immunological abnormalities play an important role in the development and progression of the disease, oxidative stress increases due to overproduction of free oxygen radicals occurring in the disease process or the effectiveness of antioxidant defense systems (1,2).

Melatonin (MLT) is a hormone secreted from the pineal gland and primarily regulates the circadian rhythm. In addition to its regulatory properties such as mood, sleep, reproductive and immune system regulation, it has antioxidant and anti-inflammatory effects (3). It has been reported that the immune system regulatory feature is in the form of pleiotropic action or buffering, since MLT can have an immune stimulating effect in basal conditions or under immunosuppressive conditions, or it may show an anti-inflammatory activity by performing an inhibitory effect in the immune system in the presence of chronic inflammation (4,5). In addition, thanks to its antioxidant and anti-inflammatory properties, it directly cleans free radicals and indirectly decreases the tissue damage that occurs during inflammation by reducing the production of agents (cytokines and adhesion molecules) that contribute to cellular damage (6). Despite this information, the immune regulatory role of MLT is very complex and its mechanisms are not yet fully understood (4). Due to these features, it has been suggested that MLT may play a role in the immunopathogenesis and etiology of many chronic inflammatory diseases and can also be used in their treatments (7). However, there is no study in the literature examining serum MLT level and the relationship between disease activity and MLT in BD.

In our study; we aimed to investigate whether there is a possible relationship between disease activity and MLT levels by determining serum MLT levels of patients with BD.

Materials and Methods

This study was conducted between November 2019 and December 2019 by Atatürk University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Division of Rheumatology. The study protocol was approved by the Atatürk University Faculty of Medicine Ethics Committee (decision no: 16, date: 07.11.2019). A written informed consent was obtained from each subject. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The study included 40 patients based on BD diagnostic criteria recommended by the international study group and 40 age-matched healthy controls (8). All patients were evaluated by the same physician.

In the study, patients with BD who meet the international study group diagnostic criteria, who have a disease activity score one and above, between 18-65 years, are included while patients with any additional systemic inflammatory or autoimmune and rheumatological diseases, acute or chronic infection, hematological disease, diabetes mellitus, history of malignancy, vision problems and drug use that would affect MLT release (antidepressant, sleep and beta blocker etc) were not included.

The participants' gender, age, body mass index (BMI), disease duration, hemogram parameters [White blood cell count (WBC), neutrophil (Neu), lymphocyte (Lym), monocyte (Mon), platelet, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), Neu/Lym ratio and platelet/Lym ratio] values, erythrocyte sedimentation rate (ESR; mm/h) and C-reactive protein (CRP; mg/mL) levels and serum MLT level (pg/mL) It was evaluated. Disease activities of the patients were evaluated using the Behçet's Disease Current Activity Form-2006 (BDCAF) score. Thirty-four patients were on colchicine, 9 were on tumor necrosis factor-alpha inhibitors, 12 were on azathioprine, 2 were on cyclophosphamide and 5 were on corticosteroid medication. In order to determine the disease activity, BDCAF, which was translated into Turkish by Hamuryudan et al. (9), was used. This form includes only the evaluation of clinical findings. In this form, which does not include pathergy test or laboratory findings, each symptom occurring according to the system affected by BD is scored and evaluated based on the duration in the last four weeks. Patients are evaluated based on symptoms such as headaches, oral ulcers, genital ulcers, skin lesions such as erythema and pustules, joint findings such as arthritis and arthralgia, and gastrointestinal system findings (nausea, vomiting, abdominal pain/diarrhea, bloody stool). It is evaluated as 0 point for absence and 1 point for presence of the symptoms. Symptoms vascular, nervous system and eye involvement are also questioned. The total score of the form is between 0-12.

Venous blood samples were taken to biochemistry and ethylenediaminetetraacetic acid hemogram tubes between 8:00-9:00 in the morning using a vacutainer after the participants rested in sitting position. As blood samples were taken for ESR and hemogram parameters measurement, their immediate transfer to the laboratory was provided, and for CRP measurements, the blood samples were stored at the room temperature for 30 minutes for coagulation and analyzed daily after centrifugation. WBC, Neu absolute count, Neu percentage (%), Lym absolute count, Lym %, Mon absolute count, Mon %, platelet count (PLT) and MPV, PDW, PCT values were recorded. Serum samples for MLT measurement were aliquoted and stored at -80 ˚C until analyzed. The analysis was performed in the Medical Biochemistry Laboratory of our hospital.

The ESR (0-20 mm/h) was measured with the Western Green method using Interrliner XN (Sysmex Corporation, Kobe, Japanese) automatic ESR analysis device and the CRP (0-5 mg/mL) was quantitatively measured with the immunoturbidometric method using Beckman Coulter AU5800 autoanalyser (Beckman Coultr Inc. Ca, USA). Sysmex XN 1000 (Sysmex Corporation,

Kobe, Japan) device was used for complete blood count. MLT levels measurement was performed by using SunLong (Cat No: SL1169Hu, Sunlung Biotech Co., Ltd., HangZhou, China) kit and measured by enzyme-linked immunosorbent assay (ELISA) method following the experimental stages according to the proposed protocol. Dynex automated ELISA reader device was used (Dynex Technologies Headquarters, Chantilly, USA).

Statistical Analysis

Power analysis for serum MLT level was performed at 95% strength and 95% confidence interval. The mean values for the MLT level were 23.6±2.5 pg/mL for the patient group and 16.4±3.7 pg/mL for the control group. Statistical analyzes were performed by using SPSS 20.0 (SPSS, Chicago IL, United States) program. Results were given as mean ± standard deviation and minimum-maximum. The suitability of the parameters to normal distribution was evaluated with the Kolmogorov-Smirnov test. The t-test (independent samples t-test, or Student's t-test) was used to compare the parameters that showed normal distribution, and the Mann-Whitney U test was used to compare parameters that did not show normal distribution. Pearson and spearman methods used for correlation analysis.

Results

A total of 40 patients (19 women and 21 men) (mean age 35.3±9 years; range: 19 to 57 years) diagnosed with BD, and a total of 40 healthy controls (mean age: 37.7±11.2 years; range: 19 to 65 years) were included in the study.

Demographic, laboratory and clinical features of the patients and healthy individuals are shown in Table 1. There was no significant difference between the other demographic characteristics and laboratory parameters, except serum MLT levels and mean MPV values ($p<0.05$). The mean disease duration in the patient group was 86.7±75.6 (range: 1-348 months) months. The mean value of BDCAF score of the patients was 3.7±1.9 (Table 1).

Data on the frequency and percentage of BDCAF clinical parameters and serum MLT levels in Behçet's patients are shown in Table 2. In the examination made according to the presence of clinical parameters used in evaluating the disease activity in BD patients; serum MLT values were found to be significantly lower in patients with headache (23.4 pg/mL) compared to patients without headache (24.6 pg/mL) ($p<0.05$). But there was no significant difference in other clinical parameters (Table 2).

Data showing the relationship between Behçet patients' laboratory parameters, disease duration, BDCAF scores and serum MLT level are shown in Table 3. No relation was found between the parameters evaluated and the BDCAF score and serum MLT levels (Table 3).

Discussion

This is the first study evaluating the relationship between MLT levels and disease activity in BD patients. In our study, serum MLT hormone levels were significantly higher in the BD group compared to the control group, but there was no significant relationship between disease activity. In addition, patients with

Table 1. Comparison of the demographic, laboratory and clinical characteristics of patients with Behçet's disease and healthy control individuals.

	Patients (n=40)	Controls (n=40)	p
Gender (f/m)	19/21	20/20	0.823
Age (mean ± SD)	35.3±9	37.7±11.2	0.289
BMI (kg/m^2)	25.4±4.3	26.8±3.3	0.111
Disease duration (month) (mean ± SD)	86.7±75.6	-	-
WBC (x10^3/µL)	8.4±2.7	7.7±1.9	0.163
Neutrophil (x10^3/µL)	5±2.3	4.9±1.7	0.734
Lymphocyte (x10^3/µL)	2.5±0.6	2.5±0.8	0.756
Monocyte (x10^3/µL)	0.7±0.2	0.6±0.2	0.227
Platelet (x10^3/µL)	293±69	285±54	0.568
MPV (fL)	10±0.8	9.6±1	0.032*
PDW (%)	11.5±1.7	12.4±2.7	0.056
PCT (%)	0.3±0.1	0.3±0.1	0.147
NLR	2.1±1	2.2±1.1	0.905
PLR	124±34	122±37	0.800
ESR [mean ± SD (minimum-maximum)] (mm/h)	11.3±9.2 (2-47)	8.2±5.9 (1-22)	0.078
CRP (mean ± SD) (mg/mL)	5.9±4.7 (3-23)	4.4±1.9 (2-10)	0.413
Serum melatonin level (mean ± SD) (pg/mL)	23.8±1.7	16.4±3.4	0.001**
BDCAF score	3.7±1.9	-	-

f: Female, m: Male, SD: Standard deviation, BMI: Body mass index, WBC: White blood cell, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, BDCAF: The Behçet's Disease Current Activity Form-2006, *$p<0.05$: Statistically significant difference between groups, **$p<0.01$: Statistically significant difference between groups

Table 2. The frequency and percentage of Behçet's Disease Current Activity Form-2006 parameters in patients with Behçet's disease

Clinical symptoms and signs		with; n (%)	without; n (%)	p
Headache		26 (65%)	14 (35%)	0.027*
Oral ulcer		26 (65%)	14 (35%)	0.943
Genital ulcer		7 (18%)	33 (82%)	0.460
Skin lesions	Erythema	16 (40%)	24 (60%)	0.987
	Pustule	25 (63%)	15 (37%)	0.912
Joint involvement	Arthralgia	29 (73%)	11 (27%)	0.175
	Arthritis	6 (15%)	34 (85%)	0.630
GIS involvement	Abdominal pain	13 (33%)	27 (67%)	0.246
	Bloody diarrhea	-	40 (100%)	-
Eye involvement (active uveitis)		1 (3%)	39 (97%)	-
Nervous system involvement		-	40 (100%)	-
Great vascular involvement		-	40 (100%)	-

n: Number of patients with Behçet's disease with symptoms or signs, %: Percentile, GIS: Gastrointestinal system
*p<0.05: Statistically significant difference between those with and without clinical symptoms and signs

Table 3. The relationship between laboratory, clinical and Behçet's Disease Current Activity Form-2006 scores and serum melatonin level in Behçet's disease patients

	r	p
WBC	0.198	0.078
Neutrophil	0.109	0.336
Lymphocyte	-0.085	0.454
Monocyte	0.207	0.066
Platelet	0.008	0.946
MPV	0.183	0.103
PDW	-0.195	0.083
PCT	0.097	0.391
NLR	0.112	0.323
PLR	0.061	0.592
ESR (mean ± SD) (mm/h)	0.202	0.070
CRP (mean ± SD) (mg/mL)	-0.059	0.602
Disease duration (mean ± SD)	0.135	0.408
BDCAF score	-0.251	0.118

WBC: White blood cell, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, BDCAF: The Behçet's Disease Current Activity Form, *Correlation is significant at the p<0.05

headache were found to have significantly lower serum MLT levels than those without headaches.

In previous studies, different laboratory parameters as well as clinical findings were used to determine the activity of BD. Although acute phase proteins, immunoglobulin, complement levels, autoantibodies, surface markers, cytokines, Lyms and many other hemogram parameters have been investigated, there is no specific laboratory marker for BD (10). However, some laboratory abnormalities associated with the disease activation can be detected, although not in all patients. It has been reported that ESR, CRP levels and Neu activation, which are among the frequently used indicators, are associated with systemic inflammation and are not significant in reflecting disease activity, but when these parameters increase in an inactive patient, they may be instructive for a detailed research (11). Platelets play role in inflammation and the cytokines and chemokines, released from activated platelet membranes, play an important role in immune response and the size of activated platelets increase. In other words, increased MPV is an indication that the platelet is activated (12). There are conflicting results about the increase or decrease of MPV values in BD, as well as its relationship with disease activity (13,14). However, it is known that patients with thrombosis have significantly higher MPV values than those who do not, and this increases the risk of deep venous thrombosis (14,15).

In the laboratory parameters evaluated in our study, there was no significant difference between the groups except for the average MPV value. The mean MPV values in our study were within normal limits in both groups (normal; 5.9-11.3 fL). However, mean MPV values were significantly higher in the patient group than in the control group. Based on our findings, we cannot say that there is an isolated MPV increase in patients with BD. This result may be derived from the low number of patients, the low disease activity and the normal ESR, CRP, WBC, PLT and other systemic inflammation parameter values. In addition, no significant correlation was found between MPV values and serum MLT levels in our study. However, our patients did not have any symptoms or signs such as pain in the arm, leg or face, swelling, discoloration, which would indicate an increased coagulative condition due to vascular involvement. This may be due to the MPV values in our patients being within normal limits.

In addition to the immune regulatory role, MLT shows anti-inflammatory and antioxidant activity by reducing the production of agents (cytokines and adhesion molecules) that contribute to cellular damage in the presence of chronic inflammatory status (4,16). It also has protective functions against vascular endothelial dysfunction in various pathological conditions due to the protecting effects on endothelial damage, vasoconstriction, platelet aggregation and leukocyte infiltration. The presence of MLT receptors throughout the body, including vascular endothelial cells and platelets, supports the ability to perform these functions (4,16). In many studies, it has been reported that MLT plays a role in the development and pathogenesis of autoimmune and/or rheumatological diseases (17,18). In addition to being involved in the regulation of the immune system and rheumatological diseases, it is also effective in reducing oxidative stress and apoptosis (7). Similar to the results of our study in many studies investigating rheumatological diseases; Increased MLT levels were found in patient groups. For example, in studies investigating the relationship between rhythmic symptoms and findings in autoimmune diseases such as rheumatoid arthritis (RA) and ankylosing spondylitis, and circadian MLT release; it was reported that serum MLT levels of patients were higher than control groups and that there was a significant relationship between disease activities and serum MLT levels, and it was reported that MLT may play a role in the pathophysiology of these diseases (19-21). However, there are studies reporting that although MLT levels are higher in RA patients compared to control groups, it is not associated with disease activity (22). In addition, there are studies reporting that serum MLT levels are higher in systemic lupus erythematosus patients than in control groups, but there are studies reporting that serum MLT levels are lower or the same compared to control groups (23-25).

In our study, serum MLT level was significantly higher in BD patients. This may be due to the deterioration of the circadian rhythm of MLT and/or the need for more antioxidant activity or anti-inflammatory activity in order to compensate chronic inflammation. However, there was no significant relationship between MLT level and disease activity in our study. This may be due to the low number of patients, the low activity of the disease, the disadvantages of the form used to assess the disease activity, or the effects of immunosuppressive therapy on MLT levels. In this context, given the other rheumatological diseases, we can say that how and where the MLT hormone plays a role in different autoimmune diseases is not well understood due to the different effects of the MLT hormone, and the immunopathological and clinical effects of the MLT hormone are not yet fully elucidated.

Headache is a common symptom in BD patients with or without neurological involvement. The presence of tension or migraine type of headache is not considered as neurological involvement (26,27). In BD, the evaluation of headache is difficult and correct diagnosis is very important since secondary headache causes can be devastating especially in neuro-BD with parenchymal involvement (28). There are studies that MLT has a role in the physiopathology of many types of headaches and is useful in the treatment of these headaches (29,30). In addition, it has been reported that increased oxidative stress and decreased antioxidant capacity trigger headaches by disrupting the brain blood flow (31).

In our study, there were no patients with symptoms or signs that could be considered as nervous system involvement according to BDCAF. However, it was determined that there were 26 patients (65%), including 18 women and 8 men with headache symptoms that are within the BDCAF criteria. Serum MLT levels were found to be significantly lower in patients with headache than those without. A lower MLT level in those with headaches may show us that the antioxidant capacity may have decreased, and this may be a trigger for headaches. For this reason, we think that MLT hormone may have a role in the physiopathology of headache in BD, but more detailed and comprehensive studies are needed. The mean age of patients with headache was 34.7 and 36.4 in patients without headache and were statistically similar. It is also known that MLT release is not affected by gender factor (32). Therefore, we can say that serum MLT levels were not affected by factors such as age and female gender. However, it has been reported that headache is more common in female gender since women are more affected by psychological stresses (33). In this sense, the presence of female domination in patients with headache in our study seems to be compatible with the literature.

Study Limitations

Limitations of our study can be listed as the timing of blood sample obtaining since the blood samples were obtained only between 8-9 am, when the MLT is the lowest, it does not fully reflect the circadian MLT hormone release, the low number of patients, and the immunosuppressive therapy.

Conclusion

Although we found that MLT plays a possible role in BD immunopathogenesis in BD patients with a headache history, there was no association between MLT level and disease activity. We suggest that further studies are needed to determine the possible role of MLT in BD and that our findings should be supported by future research.

Ethics

Ethics Committee Approval: The study protocol was approved by the Atatürk University Faculty of Medicine Ethics Committee (decision no: 16, date: 07.11.2019).

Informed Consent: A written informed consent was obtained from each subject.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K., N.Ö., Y.A., F.B., Concept: A.K., N.Ö., Design: A.K., Data Collection or Processing: A.K.,

N.Ö., Y.A., F.B., Analysis or Interpretation: A.K., N.Ö., Literature Search: A.K., Writing: A.K, N.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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