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(RESEARCH ARTICLE)

Serum in Behcet's disease determination of leptin and vitamin D levels

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Abstract

The aim of this study was to measure serum leptin and vitamin D levels in remission in Behcet's disease (BD) and to investigate the relationship between leptin and vitamin D levels with neutrophil / lymphocyte ratio (NLR) and platelet / lymphocyte ratio (PLR). This study was conducted in 20 healthy volunteers and 27 BH who were in remission. The age, sex, and body mass index values (BMI) of both groups were recorded. Venous blood samples were taken from both groups at the end of 12 h fasting. Serum leptin, vit-D, high-sensitivity C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), white blood cell (WBC), neutrophil, lymphocyte and platelet counts were measured from the obtained sera according to the kit protocols. There was no statistically significant difference between the groups in terms of hsCRP, ESR, WBC, leptin, vit-D and NLR and PLR values ($p> 0.05$). Correlation of leptin levels with NLR and PLR values in both groups were not statistically significant ($p>0.05$). The results showed that there was no relationship between the measured values and the course of the diseaseBD

Keywords: Behcet's disease; Leptin; 25-hydroxyvitamin D

1. Introduction

Behcet's disease (BD) is a chronic, recurrent, inflammatory disease that mainly involves mucocutaneous, ocular, joint, vascular and central nervous system involvement as well as other systems [1, 2]. The etiopathogenesis of BD is not completely known. However, genetic factors and human leucocyte antigen (HLA) types, infectious agents, heat shock proteins, endothelial cell dysfunction, oxidative stress, self-antigens and humoral and cellular immunity changes are implicated in the etiology of the disease [1, 3]. Activation of T cells and neutrophils plays an important role in the pathogenesis of the disease. NLR and PLR have recently been proposed to be used to measure the seriousness of inflammation in a variety of diseases, including cardiovascular diseases, malignancies, and diabetes [4]. When chronic inflammation is thought to play a role in BD, NLR and PLR are suggested to be used for acute and remission follow-up [3,4] T helper (Th) 1 response and proinflammatory cytokines such as interleukins (IL) (IL 6, IL 8, IL 17, IL 18) and tumor necrosis factor (TNF) -α are involved in the activation of T cells and neutrophils [5]. Leptin, a vasoactive polypeptide, is closely related to other cytokines which is involved in the pathogenesis of BD. Structural similarities of leptin and its receptor lead to classification as a proinflammatory cytokine [6] is why it is necessary to initiate the proinflammatory Th1 response [7]. Leptin occupies a role in the initiation of the Th1 response, while vitamin D, the immunomodulator, has the suppressive effect of the Th1 response [8, 9]. Low vitamin D levels have been reported in some autoimmune and inflammatory diseases [10,11]. However, in the recent researchs the effect of vitamin D on disease in BD, the results are contradictory [12]

There are few clinical trials evaluating serum leptin or vitamin D levels in BD. In this studies, the role of serum leptin level or vitamin D level in the pathogenesis of BD or its effect on disease activity was investigated. In the literature, there

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is no clinical study which evaluates the effect of serum leptin and vitamin D levels on the disease BD yet. In addition, there is no information about assesses the relationship between serum leptin and vitamin D levels and NLR and PLR, which are thought to be indicative of disease remission. In this study, we aimed to measure serum leptin and vitamin D levels in remission in BD and to investigate the relationship between leptin and vitamin D levels with NLR and PLR.

2. Material and methods

The present study was planned to be performed in Behcet's patients who met the diagnostic criteria of the International Behcet Disease [13] and were in the clinical remission period and in healthy volunteers. Individuals between the ages of 18-65 were included in the study. This study was conducted in 20 healthy volunteers and 27 BH who were in remission Figure 1. There were formed volunteer patients with BH Group 1; and healthy volunteers Group 2 were formed.

Patients with as clinical and laboratory active BH, patients with systemic disease other than BD (diabetes mellitus**,** hypertension, malignancy, cerebrovascular disease, coronary artery disease, infectious diseases, liver and renal failure, dyslipidemia). These people who have taken vit-D supplement, hormone replacement, corticosteroids and antiplatelet drug in the last six months, and patients with BD who received immunosuppressive and cytotoxic drug treatment outside of colchicum was excluded from the study. Age, sex, height and weight were recorded in both groups and body mass index (BMI) was calculated as weight (kg) / height (m^2) ratio.

Venose blood samples were taken from BH and healthy volunteers between 08:00- 10:00 a.m at the end of 10-12 hours of fasting. The obtained sera were stored at -20 ° C until measurements were made according to the kit protocol. Leptin measurement was measured by the ELISA method using DRG Leptin (DRG Insturument GmbH, Germany) commercial kits. Vitamin D levels were also measured using electrochemiluminescant method Roche Cobas e601, Germany). hsCRP concentrations were measured using nephelometry (Beckman Coulter, California, USA). ESH is determined using the Westergren method, WBC levels as well as neutrophil, lymphocyte and platelet levels were measured simultaneously.

The data obtained in our research were loaded into SPSS version 22.0 software (IBM Corp. Armonk, NY, USA) and the descriptive statistics were used in the evaluation of the data using the Khi square test and the kolmogrov-smirnov Z test (for normality test). Independent sample Student's t test was used for group comparisons because the data were fit to normal distribution and the error level (p) was taken as (0,05).

3. Results

Fifty patients with BD and 20 healthy volunteers were included in the study. However, 23 patients with BD who did not meet the inclusion criteria were excluded. Twenty-seven patients with BD who were involved in the study were defined as Group 1 and 20 healthy individuals as Group 2. The groups were similar in terms of age, gender and BMI (p> 0,05). The age, gender and BMI of both groups are shown in Table 1. The hsCRP, ESR and WBC values of the groups were not statistically significant (p> 0, 05). The hsCRP, ESR and WBC values of both groups are shown in Table 2. There was no statistically significant difference between leptin, vitamin D, NLR and PLR values among the groups (p> 0, 05). The leptin, vitamin D, NLR and PLR values of both groups are shown in Table 2. Correlation of leptin level with NLR and PLR values in both groups was not statistically significant (p> 0, 05). In the same way, the correlation of vitamin D level with NLR and PLR values of both groups was not statistically substantial (p> 0, 05).

Table 1 Distribution of control and patient by mean BMI and age of groups, and sex

		Behcet 's disease Average±SD	**Control Average±SD**	*** p value**
Age		38,66±12,29	41,10±15,91	0,573
BMI		25,56±4,02	25,43±6,72	0,940
Sex	Male (%)	14 (51,9)	10 (50)	0,567
	Female (%)	13 (48,1)	10 (50)	

*Statistically significant (p≤0,05) when compared with control group.; BMI: Body Mass Index; SD: Standart deviation

Table 2 Esr, Hscrp, Wbc Values In Behcet's Disease And Control Groups

	Behcet's disease Average±SD	**Control Average±SD**	***p value**
ESR	12,81±12,66	12,71±7,89	0,984
hsCRP	4,09±2,26	3,82±2,64	0,793
WBC	696,96±2730,35	7013,50±1695,02	0,942

*Statistically significant (p≤0,05) when compared with control group; hsCRP: High-sensitivity C-reactive protein;

ESR: erythrocyte sedimentation rate; WBC: White blood cell

Table 3 Vitamin D, Nlr, Plr Values In Behcet's Disease And Control Groups

	Behcet's disease Average ± SD	**Control Average ± SD**	***p value**
Leptin	11,35±8,51	19,17±16,27	0,061
Vitamin D	22,60±15,75	27,76±13,01	0,239
NLR	2,24±1,23	1,87±,69	0,230
PLR	139,10±75,93	107,03±29,06	0,053

*Statistically significant(p≤0,05) when compared with control group; NLR: neutrophil / lymphocyte ratio; PLR: platelet / lymphocyte ratio

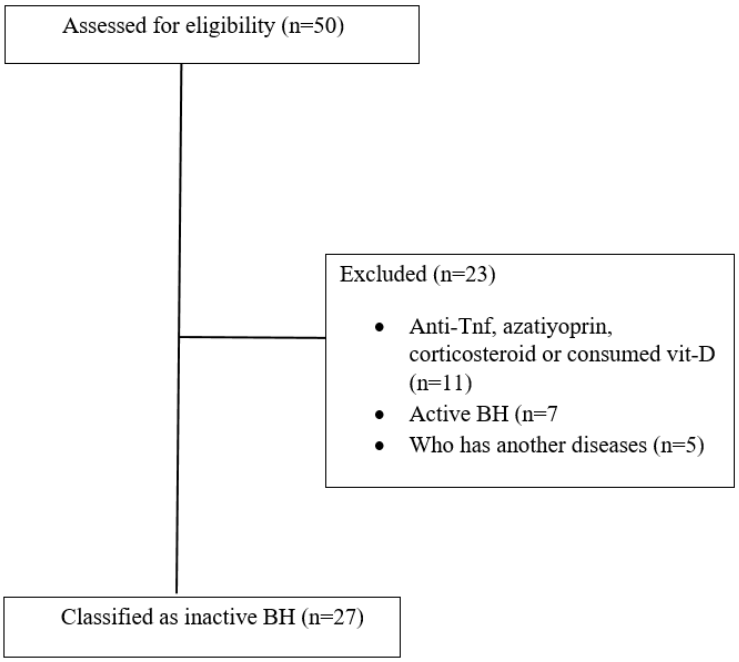

Figure 1 Flow diagram of patient selection process

4. Discussion

In this study, serum leptin and vitamin D levels were compared between patients with BD and the control group, but no statistically significant difference was found. In addition, serum NLR and PLR levels were compared between patients

with BD and in the control group. There was no statistically significant difference between serum NLR and PLR levels in both groups Table 3. These findings in our study are incompatible with previous clinical trials [14, 15]. This incompatibility may have been due to the fact that patients with BD in remission in our study were treated with active BD patients in previous clinical trials. In addition, we did not find a statistically significant correlation between serum NLR and PLR levels and serum leptin and vitamin D levels. It is first time we want to mention that these results were found by us.

Recent studies in BH have shown that cytokine adipokine producing cells also play an important role in inflammation and immunopathogenesis. Adipokines were initially described as a growth factor for liver, skeletal muscle, and bone marrow B-lymphocytes. From adipokines, leptin is first produced by adipocytes [5]. Leptin regulates body weight and appetite as well as endocrine and immunological functions. It also has impact in the different phases of the neurohumoral inflammatory response. Leptin has particular pro-inflammatory effects on natural and acquired immunity [16]. In studies done by Evereklioğlu *et al*. [17] and Yalçındağ *et al.*[18] serum leptin levels were found to be high in BD and correlated with disease activity. Furthermore, İnanır *et al.*[19] found that serum leptin levels in active and inactive BD were high. Unlike previous clinical studies in our study, serum leptin levels in patients with BD were not different from those in the control group. We think that this difference may be due to the fact that previous studies on patients with BD with remission in our study have been based on active BD patients.

It is known that vitamin D is important in the calcium and phosphorus metabolism. However, it has effects as antinflammatory and immunomodulator. Vitamin D has a suppressive role in Th1 cell response and TNF-α, interferon (IFN) -γ, IL-2, IL-5 production and has a stimulatory effect on Th2 cell differentiation, as unlike leptin [7,20]. Due to these effects of vitamin D, the number of studies evaluating vitamin D levels in BH has increased in recent years. Jassim *et al*. [21] found that vitamin D levels in BD were lower than those in the control group. In similar studies, serum vitamin D levels in BD were found to be lower than in the control groups [15, 22]. But the relationship between vitamin D level and disease activity in BH is not clear [12]. Do *et al.* [23] found that vitamin D levels in active and inactive BD were lower than those of the control group but not significantly different between active and inactive BD. It has been reported that geography, race and cultural factors may affect vitamin D levels [24,25]. In the present study, serum vitamin D levels were not different between patients with BD and the control group (Table 3). We are of the opinion that this work can only be done in patients with BD who are in remission and that the study is due to the geographical conditions and cultural structure of the region.

Laboratory tests for BH are not useful and clinical findings are based on diagnosis. However, some markers such as interleukin-6, tumor necrosis factor, ESR, CRP, thrombomodulin, E-selectin, vascular endothelial growth factor and total homocysteine have been reported as inflammatory markers in BD. It has recently been reported that blood parameters such as NLR and PLR can be used in disease activity and follow-up in BD [26, 27]. Rifaioğlu *et al*. found that NLR values in BD were higher than those in the control group [28]. In the same way Öztürk et al. found that NLR values in BD were higher in BD than in the control group [4]. Another study reported that the NLR values of both active and inactive BD were higher than those of the control group [3]. In their study of Alan *et al.*[27] they classified the disease as mild, moderate, and severe disease and PLR levels were significantly higher in the 3 groups than in the control group. Jiang *et al.* found that PLR values were significantly higher in active and inactive BD than in healthy controls, as evidenced by active BD [29]. In our study, unlike the literature, there was no difference between serum NLR and PLR values between patients with BD and control group Table 3.

We consider that this difference may be due to the fact that our work is done only in patients with remission BD. Furthermore, in our study, there was no statistically significant correlation between serum NLR and PLR levels and serum leptin and vitamin D levels in BD patients. We would like to mention that this is the first time we have done this work. There are limitations in our study because of the low number of patients, the availability of patients included in the study from a single center, and the inclusion of only inactive BD patients into the study. In addition, the hsCRP level and ESR and WBC values in patients with BD were not different from the control group in our study Table 2.

5. Conclusion

Serum leptin and vitamin D levels in inactive BD patients are not different from healthy controls, even though BD is a chronic inflammatory disease. In addition, NLR and PLR values in BD are not different from healthy controls and there is no significant correlation between serum NLR and PLR levels and serum leptin and vitamin D levels in patients with BD. However, there is requirement for further clinical trials in patients with BD to perform serum leptin and vitamin D levels, as well as multi-center, large-scale patient groups, including active and inactive BD patients, investigating NLR and PLR levels.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Statement of informed consent

If studies involve information about any individual e.g. case studies, survey, interview etc., author must write statement of informed consent as "Informed consent was obtained from all individual participants included in the study."

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