Ulusal ROMATOLOJİ Dergisi
Journal of Turkish Society for Rheumatology www.romatolojidergisi.org

Original Article / Orijinal Araştırma

DOI: 10.4274/raed.galenos.2024.77486
Ulus Romatol Derg 2025;17(1):1-8

Clinical significance of serum interleukin-32 levels in vasculo-Behçet's disease: A cross-sectional study

Vaskülo-Behçet hastalarında interlökin-32 düzeylerinin klinik önemi: Kesitsel bir çalışma

Serdar Kaymaz[1], Murat Yiğit[1], Aydın Demiray[2], Uğur Karasu[1], Veli Çobankara[1], Seçil Tan[3], Yavuz Dodurga[4]

[1]Pamukkale University Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Denizli, Türkiye
[2]Pamukkale University Faculty of Medicine, Department of Genetics, Denizli, Türkiye
[3]Pamukkale University Institute of Medical Sciences, Department of Cancer Molecular Biology, Denizli, Türkiye
[4]Pamukkale University Faculty of Medicine, Department of Medical Biology, Denizli, Türkiye

Abstract

Objective: Interleukin-32 (IL-32) is a key mediator in various pathological processes, such as the activation of vascular smooth muscle cells, the progression of atherosclerosis, and the inflammation of endothelial cells. The objective of this study was to ascertain whether Behçet's disease (BD) patients who exhibit vascular involvement have an elevation in serum levels of IL-32. Furthermore, the study aimed to explore the correlation between disease activity and IL-32 levels and in these individuals.

Methods: This cross-sectional study involved 42 patients diagnosed with BD and 38 healthy control participants, all matched for age and sex. The patients were further categorized into two groups according to whether they had vascular involvement. Comprehensive data were collected, including demographic information, disease activity, disease duration, and ongoing medical treatments. Serum levels of IL-32, IL-6, IL-17, and tumor necrosis factor (TNF)-alpha were quantified using the Enzyme-Linked ImmunoSorbent Assay method. To evaluate disease activity, two tools were utilized: the Behçet's Disease Current Activity form (BDCAF) and the Behçet's Syndrome Activity scale (BSAS).

Results: When comparing clinical features, no significant differences were observed between BD patients who had vascular involvement and those who did not have such involvement. However, vascular involvement significantly influenced the serum levels of IL-32 and TNF-alpha. Patients with BD and vascular involvement exhibited notably higher serum levels of IL-32 and TNF-alpha than healthy controls (p=0.003 and p=0.001, respectively). Furthermore, serum levels of IL-32 were significantly elevated in BD patients with vascular involvement compared to those without (p=0.008). Despite these findings, no

Öz

Amaç: İnterlökin-32'nin (IL-32) vasküler düz kas hücresi aktivasyonunda, aterosklerozda ve endotelyal enflamasyonda rolü vardır. Bu çalışmada damar tutulumu olan Behçet hastalığında (BH) serum IL-32 düzeylerinin artıp artmadığını, ayrıca IL-32 düzeyleri ile hastalık aktivitesi arasındaki ilişkiyi inceledik.

Yöntem: Bu kesitsel çalışmada 42 BD hastası ve 38 sağlıklı birey yaş ve cinsiyete göre eşleştirildi. Behçet hastalarını damar tutulumu olup olmamasına göre iki gruba ayırdık. Hastaların demografik verileri, hastalık süreleri, hastalık aktiviteleri ve tedavileri kaydedildi. Çalışılan örneklerde tümör nekroz faktörü (TNF)-alfa, IL-6, IL-17 ve IL-32'nin serum konsantrasyonlarını belirlemek için Enzime Bağlı İmmünosorbent testi tekniği kullanıldı. Behçet Sendromu Aktivite ölçeği (BSAS) ve Behçet Hastalığı Güncel Aktivite formu (BDCAF) kullanılarak hastalık aktivitesinin değerlendirilmesi yapıldı.

Bulgular: Damar tutulumu olan ve olmayan BH'nin klinik özellikleri karşılaştırıldığında anlamlı bir fark saptanmadı. Vasküler tutulumun varlığı, TNF-alfa ve IL-32'nin serum seviyelerini etkiledi. Damar tutulumu olan BH'de serum IL-32 ve TNF-alfa düzeyleri sağlıklı kontrollere göre anlamlı derecede yüksekti (sırasıyla p=0,003; p=0,001). Damar tutulumu olan Behçet hastalarında, damar tutulumu olmayan BH'ye göre serum IL-32 düzeyleri istatistiksel olarak farklıydı (sırasıyla p=0,008). Serum IL-32 seviyeleri, dönüştürülmüş BDCAF ve BSAS aktivite ölçekleri ile hiçbir ilişki göstermedi.

Sonuç: Çalışma sonuçlarımız Behçet hastalarında serum IL-32 düzeylerinin yükseldiğini ve bunun vasküler tutulumla ilişkili olabileceğini gösterdi.

Anahtar Kelimeler: Behçet hastalığı, damar tutulumu, interlökin-32

Correspondence / İletişim:
Serdar Kaymaz MD, Pamukkale University Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Denizli, Türkiye
E-mail: dr.serdarkymaz@gmail.com **ORCID ID:** orcid.org/0000-0002-6958-5436
Received / Geliş Tarihi: 22.03.2024 **Accepted / Kabul Tarihi:** 09.11.2024 **Publication Date / Yayın Tarihi:** 19.03.2025

Cite this article as / Atıf: Kaymaz S, Yiğit M, Demiray A, et al. Clinical significance of serum interleukin-32 levels in vasculo-Behçet's disease: a cross-sectional study. Ulus Romatol Derg. 2025;17(1):1-8

association was identified between disease activity and serum levels of IL-32, as measured by the BDCAF and BSAS scales.

Conclusion: The results of this study suggest that BD patients have elevated levels of serum IL-32 and such increase may be linked to the presence of vascular involvement. This highlights a potential role for IL-32 in the functional changes associated with vascular complications in BD.

Keywords: Behçet's disease, vascular involvement, interleukin-32

Introduction

Behçet's disease (BD) is characterized by a range of systemic manifestations, including persistent oral aphthous ulcers, lesions affecting the gastrointestinal system, arthritis, and complications affecting the vascular and nervous systems.[1] Among these, vascular involvement is a significant characteristic of BD, with epidemiological and clinical studies estimating its incidence to range from 6.3% to 15.3%.[2] Vascular involvement in BD is typically marked by neutrophil-predominant vasculitis, which affects all layers of the blood vessels and the vasa vasorum. In the later stages, this condition is characterized by fibrous thickening and nonspecific inflammatory infiltration.[3] Additionally, BD-associated vasculitis is closely linked to hypercoagulability, driven by excessive thrombin generation, reduced fibrinolytic activity, platelet-neutrophil aggregation, and heightened platelet activity.[4] These pathological processes collectively lead to a greater risk of thrombotic events.

Interleukin-32 (IL-32) is a cytokine involved in multiple immune processes first recognized as being secreted by natural killer (NK) cells upon IL-2 activation.[5] IL-32 is involved in regulating a range of biological activities, including cell death and cytokine production.[6-8] This cytokine is synthesized by different types of cells, including T-cells, monocytes, and NK cells. It is critical in promoting inflammation by triggering the secretion of several pro-inflammatory cytokines and chemokines, contributing to immune and inflammatory responses.[9,10] IL-32 is a key factor in driving the pro-inflammatory signaling in endothelial cells upon different stimuli, encompassing IL-1β, thrombin, lipopolysaccharides, and platelets. Under these inflammatory conditions, IL-32 levels increase significantly. According to experimental studies, silencing serum IL-32 leads to decreased synthesis of pro-inflammatory cytokines such as IL-1α, IL-6, IL-8, and intercellular adhesion molecule-1 (ICAM-1), while simultaneously enhancing the expression of thrombomodulin/CD141, an anti-inflammatory marker.[11] Additionally, IL-32 has been associated with the development of various vascular conditions. It mediates giant cell arteritis, interacts with integrins, and is a key driver of atherosclerosis progression.[12,13] Studies have further revealed that IL-32 contributes to atherosclerosis by promoting angiogenesis in endothelial cells and altering lipid profiles, thereby exacerbating disease progression.[14]

The existing literature highlights the role of cytokines in vascular endothelial injury and thrombosis formation in vasculo-BD. Inflammatory cytokines like IL-18, IL-2, IL-12, IL-6, interferon-gamma, and tumor necrosis factor (TNF)-alpha, primarily released by T helper cells, have been identified as key contributors to pathological changes in vasculo-BD. These changes include endothelial damage, systemic perivasculitis, neutrophil infiltration, and fibrinoid necrosis.[15]

However, the relationship between IL-32 and vasculo-BD has been explored in only one published study to date.[16] Building on this limited knowledge, the current study aims to examine serum levels of IL-32 in BD patients exhibiting vascular involvement. Additionally, this study seeks to establish a potential cut-off value for IL-32 that could assist in diagnosing vascular involvement in these patients.

Materials and Methods

Patients and Controls

This study had a cross-sectional design and was carried out in the Department of Rheumatology of a university hospital between January 2021 and March 2022. Sample size calculations were based on findings from Choi et al.[17]. According to their results, to achieve a statistical power of 95% and a type I error rate of 5%, at least six participants were required in each group. These calculations were based on the expected mean IL-32 levels of 1111.24 ng/mL (standard deviation = 149.59) in one group and 631.1 ng/mL (standard deviation = 120.23) in the other. For the study, 44 patients diagnosed with BD and 38 healthy controls, all matched for sex and age, were enrolled. The participants were recruited from the rheumatology outpatient clinics at the university hospital. Healthy controls were selected from blood donors registered in the hospital's blood bank, as well as university staff and their family members. All patients were diagnosed with BD referring to the most recent International Criteria for BD.[18] Following a thorough medical history assessment,

BD patients underwent a physical examination. Vasculo-BD was diagnosed in BD patients when lesions were identified in the large or small veins, aorta, or small arteries through both clinical evaluation and radiological imaging. The patients were further categorized into two groups according to whether they had involvement of vascular structures. The patterns of vascular involvement in vasculo-BD patients have been well-established in the literature.[19] All participants underwent a series of laboratory tests, including assessments of liver function, fasting plasma glucose, sedimentation rate, renal function, C-reactive protein, and complete blood count, all of which were within the normal range. The study excluded individuals with a history of antiphospholipid syndrome, high blood pressure, systemic vasculitis, blood clotting disorders, or hematological diseases.

The approval for the study was received from the local ethics committee (approval number: E-60116787-020-290434, date: 25.01.2022 - Pamukkale University Non-Interventional Clinical Research Ethics Committee). Prior to participation, all participants were informed about the study and provided written informed consent. The study adhered to the ethical guidelines set forth in the Declaration of Helsinki, ensuring the protection and rights of all BD patients participating.

Disease activity in the study was evaluated using two assessment tools: the Behçet's Syndrome Activity scale (BSAS) and the Behçet's Disease Current Activity form (BDCAF).[20,21] The BDCAF scale measures various clinical features, including oral aphthae, genital ulcers, erythema nodosum, skin pustules, diarrhea, ocular involvement, and major vessel involvement. Scores on this scale span from 0 to 12, with higher values reflecting greater disease activity. BSAS, consisting of 10 questions, quantifies the level of discomfort caused by symptoms such as mouth ulcers, genital ulcers, cutaneous lesions, and gastrointestinal, vascular, and eye involvement over the past month. Additionally, it reflects overall disease activity and the presence of current skin lesions.

Determination of Serum Cytokine Concentrations

Blood samples (3-5 milliliters) were collected from both healthy controls and patients and placed in clot activator tubes for serum separation. The samples were incubated at room temperature for 30 minutes, followed by centrifugation at 4000 rpm for 10 minutes to separate the serum. The serum samples were then stored at -80 °C for further analyses. Serum levels of TNF-alpha (Cat. no: E-EL-H0109), IL-6 (Cat. no: E-EL-H0102), IL-17 (Cat. no: E-EL-H0105), and IL-32 (Cat. no: E-EL-H0216) were determined using the Enzyme-Linked ImmunoSorbent Assay (ELISA) technique (Elabscience, USA). For cytokine quantification, the wells of the ELISA plate were prepared by adding 100 µL of standard working solution, diluted at various concentrations specified in the kit, to the first two columns of the plate. Each antibody was added in duplicate at the same concentration in both wells. After an incubation period of 90 minutes at 37 °C, 100 µL of every sample was added to the remaining wells. Following this, a biotin-labeled detection antibody solution was added to each well (100 µL per well), and the plate was left at 37 °C for 30 minutes. Following the incubation, the solution was aspirated, and the plate was washed three times with wash buffer. Next, 100 µL of working solution of horseradish peroxidase enzyme conjugate was added to each well, and the plate was incubated for an additional 30 minutes at 37 °C. The solution was aspirated again, and the plate was washed five times. Subsequently, nine microliters of substrate reagent were added to each well, and the plate was left 37 °C for 20 minutes in a dark environment. Fifty microliters of stop solution were added to each well to terminate the reaction, and the optical density of the wells was measured at 450 nm using a microplate reader. All experiments were performed in duplicate to ensure accuracy.

Statistical Analysis

Data analysis was completed with the aid of SPSS software, version 22.0, for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics were employed to depict the demographic profile of the participants. The Kolmogorov-Smirnov test was employed to assess the normality of data. Non-parametric tests were applied for non-normally distributed variables. Spearman's rank correlation was performed to evaluate the relationships between non-parametric variables. Categorical variables were analyzed at baseline using the chi-square test. For intergroup comparisons, Kruskal-Wallis variance analysis was used, followed by post hoc Bonferroni correction with the Mann-Whitney U test for pairwise comparisons. In post hoc analyses, a p-value of <0.0167 was considered statistically significant, while for all other analyses, a p-value of <0.05 was set as the threshold for statistical significance.

Results

Two patients were excluded due to meeting the exclusion criteria. One of the excluded patients had a hematologic disorder, and the other had thrombophilia. The remaining 42 patients were then divided into two groups: group 1 consisted of 21 BD patients with vascular involvement, and group 2 included 21 BD patients without vascular

involvement.

Among the patients with vascular involvement, the following conditions were observed: nine (43%) had deep vein thrombosis, four (20%) had pulmonary embolism, three (15%) had thrombus formation in the jugular vein, three (15%) had thrombosis of the intracranial venous sinuses, two (10%) had occluded retinal vein, two (8%) had thrombophlebitis, one (5%) had portal vein thrombosis (Table 1).

The mean age of group 1 (patients with vascular involvement) was 36.8±5.0 years, with 8 females, while the mean age of group 2 (patients without vascular involvement) was 39.1±9.0 years, with 12 females. The mean disease duration was 4.7±8.7 years in group 1 and 4.7±5.8 years in group 2. The two groups showed no significant differences in demographic or clinical characteristics (Table 1).

Significant differences were observed in the serum levels of IL-32, TNF-alpha, and IL-6 between the three groups (p=0.012, p=0.021, p=0.037, respectively) (Figure 1, Table 2). BD patients exhibiting vascular complications had significantly lower serum IL-32 and TNF-alpha levels than healthy participants (p=0.003, p=0.001, respectively). Additionally, serum IL-32 levels were significantly higher in the patients with vascular manifestations compared to those without vascular complications (p=0.008) (Table 1).

However, the presence of vascular involvement did not significantly influence the levels of IL-17 (p>0.05) (Table 2, Figure 1). Furthermore, serum levels of IL-32 did not correlate with overall disease activity (Table 3).

Discussion

The findings of this study revealed significantly elevated serum concentrations of IL-32 in patients with vasculo-BD compared to BD patients without vascular complications and healthy controls. This suggests that IL-32 might be implicated in the vascular manifestations of this disease. However, it is noteworthy that the elevated IL-32 levels did not correlate with disease activity, indicating that IL-32 may be more closely associated with vascular involvement rather than overall disease severity. To the best of our knowledge, this study is one of the first to explore the relationship between serum IL-32 levels and vascular involvement in BD patients. While previous research has linked various cytokines with vascular damage and thrombosis in BD, the function of IL-32 in this context has not been thoroughly studied.

Few studies exploring the connection between IL-32 and vascular pathologies exist in the literature.

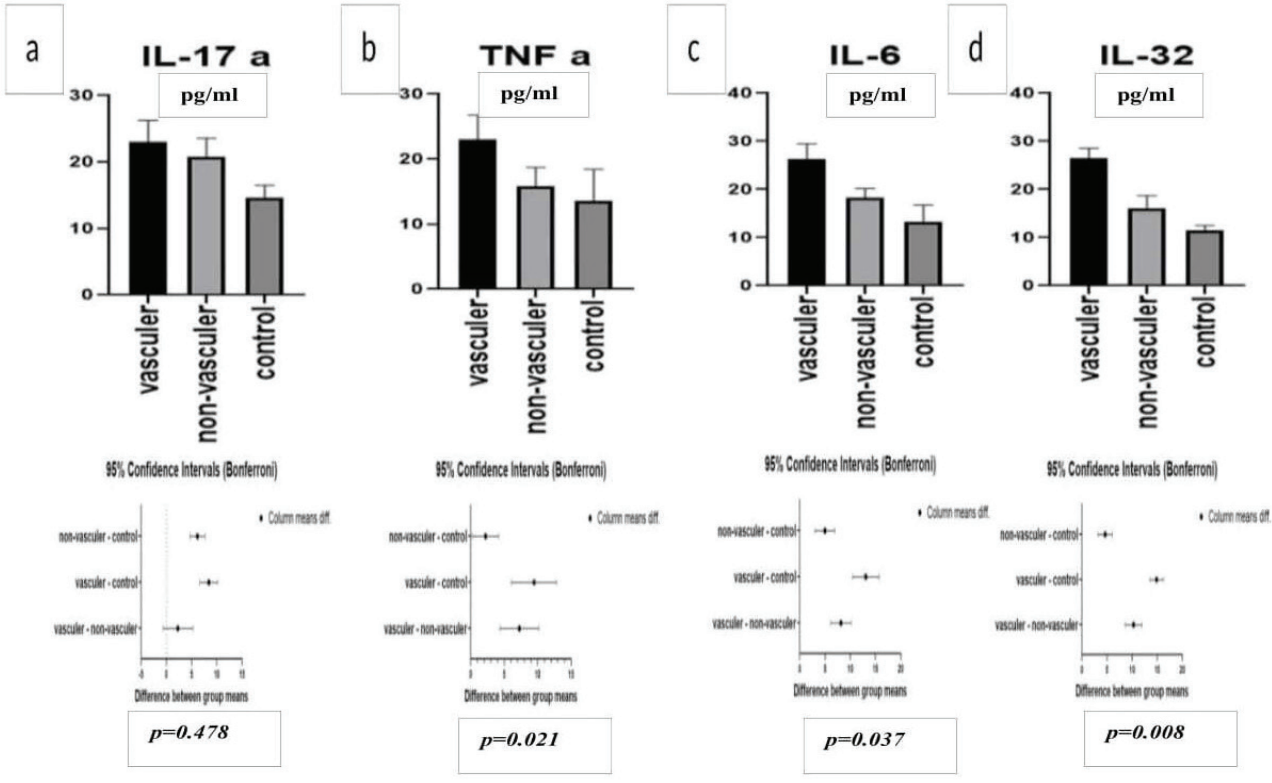

Figure 1. Serum levels of IL-32 in the study groups
IL: Interleukin, TNF: Tumor necrosis factor

Table 1. Demographic variables of participants and comparison of clinical characteristics of the groups of BD patients and medications

Mean ± SD or n (%)	Group 1 (n=21) BD with vascular involvement	Group 2 (n=21) BD without vascular involvement	Group 3 (n=38) healthy controls	p-value
Gender				
-Male	13 (62)	9 (43)	17 (45)	0.369
-Female	8 (38)	12 (57)	21 (55)	
Age (year)	36.8±5	39.1±9	37.8±6.5	0.518
Disease duration (year)	8.7±4.7	4.7±5.8	-	0.827
Laboratory findings				
-CRP	0.33±0.48	0.66±0.79		0.109
-Sedimentation	20±8.4	24±8.2	-	0.120
-HLA-51 positivity	14 (66)	16 (76)	-	0.5
Medical treatment				
-Steroid	4 (19)	5 (24)		0.799
-Colchicine	10 (48)	12 (59)		
-Azathioprine	3 (14)	4 (19)		
-Cyclosporine	5 (24)	2 (9.5)		
-Methotrexate	1 (5)	4 (19)		
-Interferon	1 (5)	1 (5)		
-Biologic agents	8 (38)	6 (28.5)		
-Anticoagulant	10 (50)	-		
Clinical lesions				
-Oral lesions	18 (86)	15 (71)	-	0.970
-Ocular	4 (20)	6 (30)	-	
-Articular	2 (10)	2 (10)	-	
-Pulmonary	1 (5)	2 (10)	-	
-Neurological	1 (5)	1 (5)		
Disease activity				
-BDCAF	0.4±0.9	0.19±0.5	-	0.224
-BSAS	1.9±1	1.9±0.7	-	0.99

-Kruskal-Wallis and Mann-Whitney U tests were used.

**: p<0.05 statistically significant, **: p<0.0167 statistically significant in the post hoc Bonferroni correction analyses, BD: Behçet's disease, BDCAF: Behcet's Disease Current Activity form, BSAS: Behçet's Syndrome Activity scale, CRP: C-reactive protein: HLA: Human leukocyte antigen, SD: Standard deviation*

Table 2. Comparison of serum levels of cytokines

Mean ± SD	Group 1 (n=21) BD with vascular involvement	Group 2 (n=21) BD without vascular involvement	Group 3 (n=38) healthy controls	p-value	Mann-Whitney U test with Bonferroni correction
TNF-alpha, pg/mL	16.0±5.2	15.3±7.6	11.7±2.3	**0.021***	Group 3<Group 2, p=0.012** Group 2=Group 1, p=0.402 Group 3<Group 1, p=0.001**
-IL-6, pg/mL	8.6±1.5	2.1±3.3	1.4±1.1	**0.037***	Group 3=Group 2, p=0.384 Group 2=Group 1, p=0.084 Group 3<Group 1, p=0.008
-1L-17, pg/mL	39.2±9.0	21.6±1.8	20.8±2.1	0.478	Group 1=Group 2, p=0.210 Group 2=Group 3, p=0.693 Group 1=Group 3, p=0.380
-1L-32, pg/mL	99.1±2.7	91.4±2.0	1.2±6.2	**0.012***	Group 1>Group 2, p=0.008** Group 2>Group 3, p=0.0160 Group 1>Group 3, p=0.003**

-Kruskal-Wallis and Mann-Whitney U tests were used.

**: p<0.05 statistically significant, **: p<0.0167 statistically significant in the post hoc Bonferroni correction analyses, BD: Behçet's disease, IL: Interleukin, SD: Standard deviation, TNF: Tumor necrosis factor*

Table 3. Correlation of serum IL-32 levels with disease activity scales and other cytokines in BD patients with vascular involvements

	IL-32	
	r	p
TNF-alpha	-0.694	0.103
IL-6	-0.112	0.481
IL-17	-0.150	0.345
BSAS	0.120	0.243
BDCF	0.075	0.637

**p<0.05, statistically significant, BD: Behçet's disease, BDCF: Behçet's Disease Current Activity form, BSAS: Behçet's Syndrome Activity scale, IL: Interleukin, TNF: Tumor necrosis factor*

Son et al.[22] demonstrated that IL-32 inhibited endothelial inflammation, atherosclerosis, and the expansion of vascular smooth muscle cells, suggesting its potential protective role in vascular health. Similarly, Kobayashi et al.[23] found that IL-32 is critical in leukocyte adhesion and endothelial inflammation. It achieves this through the enhancement of ICAM-1, vascular cell adhesion molecule, and E-selectin expression on endothelial cells, all of which are important markers of inflammation and vascular injury. These findings support the hypothesis that IL-32 might be involved in the development of vascular diseases, including vasculo-BD. The increase in inflammation and leukocyte recruitment is driven by IL-32-mediated upregulation of ICAM-1 on endothelial cells, highlighting its significance in the pathogenesis of vascular diseases such as abdominal aortic aneurysms.[24] Additionally, IL-32 has been demonstrated to regulate the functions of endothelial cells in various circulatory systems, including the aortic, pulmonary, and coronary circulations. This regulation occurs through the modulation of IL-1β and other pro-inflammatory cytokines, particularly influencing the expression of ICAM.[11]

In the current study, the significantly elevated serum concentration of IL-32 in BD patients with vascular involvement, compared to those without, may suggest that IL-32 plays a relatively peripheral role in the development of vascular pathologies in BD. However, to validate this observation and better understand the underlying mechanisms, further studies are necessary.

Despite numerous efforts over several decades, no cytokine or biomarker has demonstrated sufficient sensitivity and specificity to reliably predict vascular involvement in patients with BD. However, certain markers have shown promise. Ibrahim et al.[25] suggested that monocyte chemoattractant protein-1 and vascular endothelial growth factor (VEGF) could be valuable biomarkers for thrombosis prediction in patients presenting with BD. The same study highlighted that VEGF contributes to endothelial and tissue damage by increasing the release of free radicals through nitric oxide production, a condition that has been linked to thrombosis in BD.

In addition, other studies have identified alterations in specific biomarkers associated with vascular involvement in BD. For instance, one study reported that serum angiopoietin-1 concentrations were significantly lower in patients with BD exhibiting vascular manifestations compared to those without vascular complications, suggesting its potential role in vascular pathology.[26] These findings reinforce the need for continued exploration of biomarkers to better predict and understand vascular involvement in BD. It is well established that angiopoietin-1 indirectly influences angiogenesis through the regulation of VEGF.[27] Several cytokines like TNF-alpha, IL-32, IL-6, and IL-1 are involved in the regulation of VEGF.[25,28] This study showed significantly different IL-32 levels between BD patients with and without vascular complications. This finding suggests that IL-32 could serve as a promising biomarker for diagnosing or predicting vascular involvement in BD, offering potential clinical value.

While these results are promising and may help guide clinicians in practice, our understanding of the exact mechanisms linking IL-32 to vascular involvement remains incomplete. Further research is needed to clarify the potential of IL-32 and its interactions with other cytokines and pathways involved in vascular pathology.

The link between IL-32 and various medical treatments, inflammatory cytokines, and disease activity has been explored in several studies. For instance, it has been demonstrated that IL-32 released from pulmonary cells infected with influenza A can be inhibited by aspirin or selective COX-2 inhibitors.[29] In contrast, Kwon et al.[30] found that corticosteroid inhalers did not affect IL-32 concentrations in asthma patients. Bengts et al.[24] also reported that statins did have an effect on IL-32 concentrations.

While studies examining the relationship between TNF-alpha inhibitors and serum IL-32 levels are limited, some important findings have been reported. Specifically, a critical relationship exists between TNF-alpha, a cytokine central to the onset and progression of rheumatoid arthritis (RA), and IL-32 release.[31] Hong et al.[32] reported that the suppression of serum IL-32 led to decreased TNF-alpha levels in human macrophages, providing further evidence of the interaction between these two cytokines. These findings highlight the complex interplay between IL-32 and various inflammatory mediators, suggesting potential therapeutic implications for modulating IL-32 in inflammatory diseases. Fadaei et al.[33] reported a direct relationship between IL-32, TNF-alpha, and IL-6 in patients with Diabetes Mellitus. Similarly, another study showed that IL-32 enhances IL-

17 expression in $CD4^+$ T-cells.[34] Interestingly, the same publication indicated that these cytokines serve as predictors of coronary artery disease.[35] Based on these findings, one could hypothesize that IL-32 may play a significant role in the pathogenesis of cardiovascular diseases in individuals suffering from persistent inflammatory disorders.

However, studies specifically examining the connection between disease activity and IL-32 levels in BD patients are limited. Ha et al.[16] found only a weak relationship between BDCAF and IL-32 levels. This suggests that while IL-32 may be involved in the inflammatory processes of BD, its direct role in disease activity remains uncertain and warrants further investigation. Moreover, a relationship between disease activity and IL-32 has been observed in two published studies on RA and neuromyelitis optica.[36,37] However, the results of our study did not show any correlation between IL-32 levels and disease activity scales in BD patients. The lack of correlation in our study may be attributed to several factors, such as the small number of participants, the potential influence of medications on serum cytokine levels, and the cross-sectional design of the study. To better understand the potential impact of disease activity and medication on IL-32 levels, Future studies with expanded sample sizes are needed to corroborate these results.

There are various scales available in the literature to assess BD activation, such as BDCAF and BSAS.[38] Our study revealed no correlation between serum IL-32 concentrations and BSAS or BDCAF. This lack of correlation may be attributed to the fact that these scales evaluate a broad range of organ involvement, rather than specifically focusing on vascular involvement. Buzatu et al.[39] highlighted that the Birmingham Vasculitis Activity score, which is specifically designed to evaluate vascular involvement in BD, is more sensitive than BDCAF. Therefore, the absence of a vascular-specific scale in our study could be considered a limitation, and future studies should consider using a more targeted vascular activity scale to assess the link between IL-32 and vascular involvement in BD.

Study Limitations

Our study has three potential limitations. First, we did not exclude common conditions such as smoking and hyperlipidemia, which could also influence IL-32 levels. Second, the study's cross-sectional nature limited our capacity to determine causal relationships. While it demonstrated a relationship between vascular involvement and serum concentrations of IL-32, it could not determine if elevated IL-32 levels directly cause vascular involvement in BD patients. Third, we were unable to assess a specific cut-off value for IL-32 to diagnose vascular involvement due to the absence of an appropriate diseased control group. These limitations highlight the need for further studies with more comprehensive designs to better understand the role of IL-32 in BD and its potential as a diagnostic biomarker.

Conclusion

In conclusion, the present study indicated that serum IL-32 levels were higher in BD patients with vascular involvement. Based on these findings, IL-32 might have a subtle role in the immunopathogenesis of vascular involvement in BD. However, IL-32 was not found to be associated with disease activity. Further studies are needed to confirm these results and better understand the underlying mechanisms of IL-32 in BD, particularly in relation to vascular involvement.

Ethics

Ethics Committee Approval: The approval for the study was received from the local ethics committee (approval number: E-60116787-020-290434, date: 25.01.2022 - Pamukkale University Non-Interventional Clinical Research Ethics Committee).

Informed Consent: Prior to participation, all participants were informed about the study and provided written informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Concept: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Design: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Data Collection and Processing: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Analysis or Interpretation: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Literature Search: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D., Writing: S.K., M.Y., A.D., U.K., V.Ç., S.T., Y.D.

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

Financial Disclosure: The authors declare that they have no relevant financial disclosures.

References

1. Hatemi G, Yazici Y, Yazici H. Behçet's syndrome. Rheum Dis Clin North Am. 2013;39:245-61.
2. Ishigatsubo Y. Statement draft for clinical guidelines of vasculo-Behçet's disease. [Internet]. 2014 [cited 2018 Jun. 6] Available from: http://www.nanbyou.or.jp/upload_files/Bechet2014_4.pdf (in Japanese).

3. Seyahi E, Yurdakul S. Behçet's syndrome and thrombosis. Mediterr J Hematol Infect Dis. 2011;3:e2011026.
4. Ferrão C, Almeida I, Marinho A, Vasconcelos C, Correia JA. A nossa regra de ouro na doença de Behçet: tratar a manifestação clínica. Arq Med. 2015;29:75-9.
5. Dahl CA, Schall RP, He HL, Cairns JS. Identification of a novel gene expressed in activated natural killer cells and T cells. J Immunol. 1992;148:597-603.
6. Netea MG, Lewis EC, Azam T, et al. Interleukin-32 induces the differentiation of monocytes into macrophage-like cells. Proc Natl Acad Sci U S A. 2008;105:3515-20.
7. Kang JW, Choi SC, Cho MC, et al. A proinflammatory cytokine interleukin-32beta promotes the production of an anti-inflammatory cytokine interleukin-10. Immunology. 2009;128(1 Suppl):e532-40.
8. Khawar B, Abbasi MH, Sheikh N. A panoramic spectrum of complex interplay between the immune system and IL-32 during pathogenesis of various systemic infections and inflammation. Eur J Med Res 2015;20:7.
9. Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA. Interleukin-32: a cytokine and inducer of TNFalpha. Immunity. 2005;22:131-42.
10. Netea MG, Azam T, Ferwerda G, et al. IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1beta and IL-6 production through a caspase 1-dependent mechanism. Proc Natl Acad Sci U S A. 2005;102:16309-14.
11. Nold-Petry CA, Nold MF, Zepp JA, Kim SH, Voelkel NF, Dinarello CA. IL-32-dependent effects of IL-1beta on endothelial cell functions. Proc Natl Acad Sci U S A. 2009;106:3883-8.
12. Heinhuis B, Koenders MI, van den Berg WB, Netea MG, Dinarello CA, Joosten LA. Interleukin 32 (IL-32) contains a typical α-helix bundle structure that resembles focal adhesion targeting region of focal adhesion kinase-1. J Biol Chem. 2012;287:5733-43.
13. Ciccia F, Alessandro R, Rizzo A, et al. Expression of interleukin-32 in the inflamed arteries of patients with giant cell arteritis. Arthritis Rheum. 2011;63:2097-104.
14. Yang Z, Shi L, Xue Y, et al. Interleukin-32 increases in coronary arteries and plasma from patients with coronary artery disease. Clin Chim Acta. 2019;497:104-9.
15. de Vargas RM, da Cruz MLN, Giarllarielli MPH, et al. Vascular involvement in Behçet's disease: the immunopathological process. J Vasc Bras. 2021;20:e20200170.
16. Ha YJ, Park JS, Kang MI, Lee SK, Park YB, Lee SW. Increased serum interleukin-32 levels in patients with Behçet's disease. Int J Rheum Dis. 2018;21:2167-74.
17. Choi YS, Kim S, Oh YS, Cho S, Hoon Kim S. Elevated serum interleukin-32 levels in patients with endometriosis: a cross-sectional study. Am J Reprod Immunol. 2019;82:e13149.
18. International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD). The International Criteria for Behçet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. J Eur Acad Dermatol Venereol. 2014;28:338-47.
19. Fei Y, Li X, Lin S, et al. Major vascular involvement in Behçet's disease: a retrospective study of 796 patients. Clin Rheumatol. 2013;32:845-52.
20. Hatemi G, Merkel PA, Hamuryudan V, et al. Outcome measures used in clinical trials for Behçet syndrome: a systematic review. J Rheumatol. 2014;41:599-612.
21. Lawton G, Bhakta BB, Chamberlain MA, Tennant A. The Behcet's disease activity index. Rheumatology (Oxford). 2004;43:73-8.
22. Son DJ, Jung YY, Seo YS, et al. Interleukin-32α inhibits endothelial inflammation, vascular smooth muscle cell activation, and atherosclerosis by upregulating timp3 and reck through suppressing microRNA-205 biogenesis. Theranostics. 2017;7:2186-203.
23. Kobayashi H, Yazlovitskaya EM, Lin PC. Interleukin-32 positively regulates radiation-induced vascular inflammation. Int J Radiat Oncol Biol Phys. 2009;74:1573-9.
24. Bengts S, Shamoun L, Kunath A, et al. Altered IL-32 signaling in abdominal aortic aneurysm. J Vasc Res. 2020;57:236-44.
25. Ibrahim SE, Elshishtawy HF, HelmySamy A, Galal ZA. Role of vascular endothelial growth factor and monocyte chemoattractant protein-1 in Behçet's disease. Indian J Rheumatol. 2011;6:168-72.
26. Bassyouni IH, Sharaf M, Wali IE, Mansour HM. Clinical significance of angiopoietin-1 in Behcet's disease patients with vascular involvement. Heart Vessels. 2016;31:918-24.
27. Kanazawa H. VEGF, angiopoietin-1 and -2 in bronchial asthma: new molecular targets in airway angiogenesis and microvascular remodeling. Recent Pat Inflamm Allergy Drug Discov 2007;1:1-8.
28. Zhao WB, Wang QL, Xu YT, Xu SF, Qiu Y, Zhu F. Overexpression of interleukin-32α promotes invasion by modulating VEGF in hepatocellular carcinoma. Oncol Rep. 2018;39:1155-62.
29. Li W, Liu Y, Mukhtar MM, et al. Activation of interleukin-32 pro-inflammatory pathway in response to influenza A virus infection. PloS One. 2008;3:e1985.
30. Kwon JW, Chang HS, Heo JS, et al. Characteristics of asthmatics with detectable IL-32γ in induced sputum. Respir Med. 2017;129:85-90.
31. Damen MSMA, Schraa K, Tweehuysen L, et al. Genetic variant in IL-32 is associated with the ex vivo cytokine production of anti-TNF treated PBMCs from rheumatoid arthritis patients. Sci Rep. 2018;8:14050.
32. Hong J, Bae S, Kang Y, et al. Suppressing IL-32 in monocytes impairs the induction of the proinflammatory cytokines TNFalpha and IL-1beta. Cytokine. 2010;49:171-6.
33. Fadaei R, Bagheri N, Heidarian E, et al. Serum levels of IL-32 in patients with type 2 diabetes mellitus and its relationship with TNF-α and IL-6. Cytokine. 2020;125:154832.
34. Moon YM, Yoon BY, Her YM, et al. IL-32 and IL-17 interact and have the potential to aggravate osteoclastogenesis in rheumatoid arthritis. Arthritis Res Ther. 2012;14:R246.
35. Mohammad-Rezaei M, Ahmadi R, Rafiei A, et al. Serum levels of IL-32 in patients with coronary artery disease and its relationship with the serum levels of IL-6 and TNF-α. Mol Biol Rep. 2021;48:4263-71.
36. Gui M, Zhang H, Zhong K, Li Y, Sun J, Wang L. Clinical significance of interleukin-32 expression in patients with rheumatoid arthritis. Asian Pac J Allergy Immunol. 2013;31:73-8.
37. Wang H, Wang K, Wang C, Xu F, Qiu W, Hu X. Increased plasma interleukin-32 expression in patients with neuromyelitis optica. J Clin Immunol. 2013;33:666-70.
38. Kaymaz S, Yilmaz H, Ufuk F, et al. Ultrasonographic measurement of the vascular wall thickness and intima-media thickness in patients with Behçet's disease with symptoms or signs of vascular involvement: a cross-sectional study. Arch Rheumatol. 2021;14;36:258-66.
39. Buzatu C, Duffield S, Chadwick L, Moots RJ. THU0554 the clinical utility of two vasculitis activity scores (BVAS and BDCAF) in Behçet's Syndrome: a prospective cohort study. Ann Rheum Dis. 2019;78:567.