Original Research

Turk J Immunol 2021;9(2):80-5
DOI: 10.5222/TJI.2021.03521

mRNA Expression Levels of NKp30, NKp46, NKG2D, Perforin and Granzyme in the Behçet's Disease

Behçet Hastalarında NKp30, NKp46, NKG2D, Perforin ve Granzim mRNA Ekspresyon Düzeyleri

Metin Yusuf Gelmez[1], Suzan Çınar[1], Esin Aktaş[1], Fatma Betül Öktelik[1], Goncagül Babuna-Kobaner[2], Murat Erdugan[3], Ahmet Gül[3], Afet Akdağ Köse[2], Günnur Deniz[1]

[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey
[2]Istanbul University, Istanbul Faculty of Medicine, Department of Dermatology and Venereology, Istanbul, Turkey
[3]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey

Cite as: Gelmez MY, Çınar S, Aktaş E, Öktelik FB, Babuna-Kobaner G, Erdugan M, Gül A, Akdağ Köse A, Deniz G. mRNA expression levels of NKp30, NKp46, NKG2D, perforin and granzyme in the Behçet's disease, Turk J Immunol 2021;9(2):80-5.

Received: 23.03.2021 **Accepted:** 27.05.2021 **Publication date:** 30.08.2021

Corresponding Author: Günnur Deniz, Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Vakif Gureba Cad. Fatih, Istanbul - Turkey
gdeniz@istanbul.edu.tr

Abstract

Objective: There are limited studies about NK cells that may have an immunoregulatory role in Behçet's Disease. NK cell receptor, *perforin* and *granzyme* mRNA expressions were analyzed in both active and inactive BD patients.
Materials and Methods: *NKp30, NKp46, NKG2D, perforin* and *granzyme* mRNA expression were analyzed in 42 patients with Behçet's disease and 14 controls by RT-PCR. $CD3^+$ T, and NK cell subsets were analyzed by flow cytometry.
Results: *NKp30, NKp46, NKG2D, perforin* and *granzyme* expression levels between patients and healthy subjects did not show any significant differences. However, a positive correlation was detected between $CD3^-CD16^+CD56^{dim}$ NK cell frequencies and *NKG2D* and *NKp46* mRNA levels in active BD patients and between $CD3^+$ T cell frequencies and NKp46 mRNA levels in inactive Behçet's patients.
Conclusion: Although, a significant difference does not exist between *NKp30, NKp46, NKG2D, perforin* and *granzyme* mRNA expressions, further functional studies are necessary to rule out the potential role of these molecules in the pathogenesis of Behçet's Disease.

Keywords: Behçet's Disease, NK cell, NK receptor, *NKp30, NKp46, NKG2D, perforin, granzyme*

Öz

Amaç: Behçet Hastalığında (BD) Doğal Katil (NK) hücrelerinin immünregülatör etkisine yönelik sınırlı sayıda çalışma vardır. Bu çalışmada, aktif ve inaktif Behçet hastalarında *NKp30, NKp46, NKG2D, perforin* ve *granzim* mRNA ekspresyonları incelenmiştir.
Gereç ve Yöntem: Çalışmaya 42 Behçet hastası ve 14 sağlıklı kontrol dahil edildi. *NKp30, NKp46, NKG2D, perforin* ve *granzim* mRNA ekspresyon düzeyleri RT-PCR ile, $CD3^+$ T ve NK hücreleri akan hücre ölçer ile analiz edildi.
Bulgular: Hasta ve sağlıklı kontroller arasında *NKp30, NKp46, NKG2D, perforin* ve *granzim* mRNA ekspresyon düzeyleri açısından anlamlı bir fark saptanmadı. Aktif hastalarda $CD3^-CD16^+CD56^{dim}$ NK hücre oranları ile *NKG2D* ve *NKp46* mRNA ekspresyon seviyeleri arasında, inaktif hastalarda ise $CD3^+$ T hücre oranları ile *NKp46* mRNA ekspresyonları arasında pozitif korelasyon saptandı.
Sonuç: *NKp30, NKp46, NKG2D, perforin* ve *granzim* mRNA ekspresyonları açısından anlamlı fark bulunmamakla birlikte bu moleküllerin Behçet patogenezindeki potansiyel rolünü dışlamak için daha fazla fonksiyonel çalışma gereklidir.

Anahtar kelimeler: Behçet Hastalığı, NK, NK reseptör, *NKp30, NKp46, NKG2D, perforin, granzim*

ORCID: M.Y. Gelmez 0000-0002-5279-0855, **S. Çınar** 0000-0002-8330-7010, **E. Aktaş** 0000-0002-8078-2780, **F. B. Öktelik** 0000-0002-7994-5618, **G. Babuna-Kobaner** 0000-0002-0985-5027, **M. Erdugan** 0000-0002-8942-2124, **A. Gül** 0000-0001-8219-3720, **A. Aktağ Köse** 0000-0003-3929-730X, **G. Deniz** 0000-0002-0721-6213

Introduction

Behçet's Disease that was first described by Hulusi Behçet in 1937, is seen more prevalently in silk road countries extending from Turkey to Korea and Japan.[1] Studies carried out by scientists working in our country and especially in our university have made significant contributions to the pathogenesis and clinic of the disease.[2,3] It is reported that genetic, environmental, and immunological factors play a role in the pathogenesis of Behçet's disease.[4] Mechanisms triggered by some common immunopathogenic pathways, especially through MHC-I alleles, are thought to have a role.[4,5]

Natural killer (NK) cells are derived from bone marrow and are the members of the innate immune system. NK cells target and kill infected cells with viruses or intracellular bacteria and cancer cells that try to escape from the immune system by not expressing MHC-I molecules.[6] NK cell display cytotoxic activity through perforin and granzyme molecules that induce target cell apoptosis. NK cells have also stimulating effect on other immune system cells through the secreting cytokines like interferon-gamma (IFN-γ), interleukin (IL)-17.[7,8] *NKp30, NKp44, NKp46* were first identified NK cell receptors (NCRs) during the human NK cell lysis experiments performed by Moretta et al.[9] After this discovery, NCR such as NKG2D and KIR receptor family were discovered.[10,11] These receptors are classified as activator receptors that increase NK cell function and inhibitory receptors that decrease NK cell function. *NKp30, NKp44, NKp46*, and NKG2D are activator receptors of NK cells and stimulated the cytotoxic function of NK cells and cytokine release.[9,12]

The role of NK cells has not been clarified in the pathogenesis of Behçet's disease.[4] In this study, mRNA levels of NCR and cytotoxic enzymes were analyzed in patients with Behçet's disease.

Materials and Methods

Patient Group

The study included 42 patients with Behçet's disease who were followed up in the outpatient clinics of Istanbul University, Istanbul Faculty of Medicine, Departments of Internal Diseases, Dermatology and Rheumatology.

Fourteen age and gender-matched healthy subjects that have no history of cancer or autoimmunity in themselves or their first-degree relatives, no infection at the time of the study were taken to the control group. The patients and healthy subjects who participated in the study signed the consent form of the Istanbul University Faculty of Medicine Clinical Research Ethics Committee.

Analysis of T Lymphocytes and NK Cells by Flow Cytometry

The ratios of $CD3^+$ T, $CD3^-CD16^+CD56^{dim}$, and $CD3^-CD16^-CD56^{bright}$ NK cells were detected by the whole blood lysis method using anti-CD3 PECY7 (clone UCTH1), -CD16 APC.CY7 (clone B73.1) and -CD56 AlexaFlour700 (clone 5.1H1) monoclonal antibodies that were obtained from BioLegend firm (San Diego, Ca, USA). Following the incubation of peripheral blood samples (10^6 cells/ml) in the dark for 20 minutes with the appropriate amount of monoclonal antibody, lysing solution (2 ml, x1, BD-Bioscience, USA) was added to the samples and incubated for 20 minutes at room temperature. After lysis of erythrocytes, cells were washed twice with phosphate -buffered saline (PBS) solution at 1800 rpm for 5 minutes. Data were recorded with the flow cytometry FACSAria II (Becton-Dickinson, USA) and analyzes were made with Diva software (Becton-Dickinson, USA) In all samples, 30,000 cells were counted in the lymphocyte gate on the SSC / FSC graph. $CD3^+$ T, $CD3^-CD16^+CD56^{dim}$ and $CD3^-CD16^-CD56^{bright}$ NK cell subsets were analyzed in the lymphocyte population of patients and healthy subjects.

Quantitative Real - Time (Real-Time) Polymerase Chain Reaction (qRT-PCR)

Total RNA isolation was performed with the Trizol method from peripheral blood samples and, the amount of RNA in the samples was determined by the NanoDrop spectrophotometer. From the total RNA samples, cDNA was performed by the Transcriptor High Fidelity cDNA synthesis kit (Roche, Germany) according to the manufacturer's protocol. mRNA expressions of *NKp30, NKp46, NKG2D, perforin*, and *granzyme* were analyzed by the qRT-PCR method using (Real-Time) Ready Assay (Roche, Germany) sequences specific to gene regions and LightCycler Probe 480 Master mix (Roche, Germany) kit. In the normalization of *NKp30, NKp46, NKG2D*, *perforin*, and *granzyme* data, the GADPH gene was used as a reference. The samples were rerun in duplicate and the expression levels were determined by the $2^{-\Delta\Delta CT}$ method.[13]

Statistical Analysis

Nonparametric tests (Kruskall-Wallis, Mann-Whitney U) with the SPSS 21 program were used for analyses. Pearson's correlation test was used for the correlation analysis. A statistically significant difference was accepted as $p \leq 0.05$.

Results

Study Populations

A total of 42 patients with active or inactive Behçet's disease who were not under immunosuppressive therapy

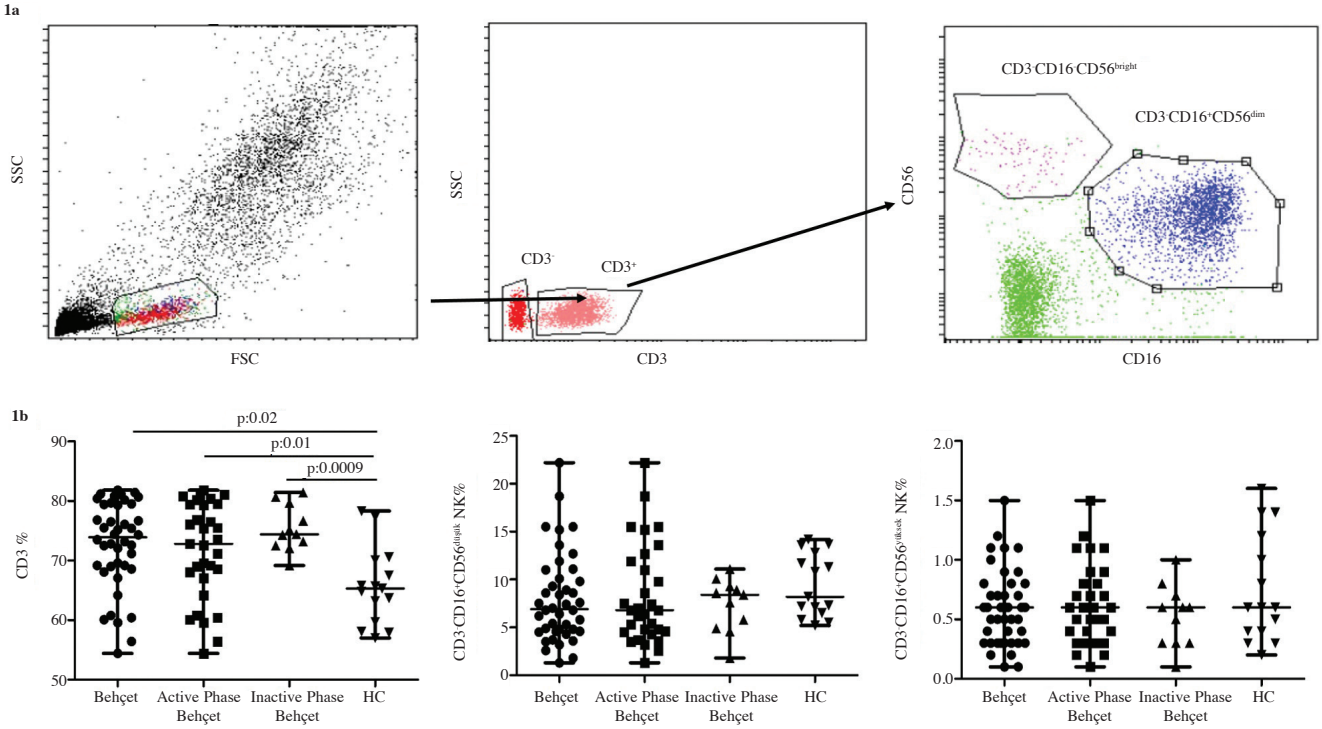

Figure 1. Gating strategy of lymphocyte, T cell and NK cells (1a). Comparison of T ($CD3^+$) and NK cell subsets ($CD3^-CD16^+CD56^{dim}$ NK and $CD3^-CD16^-CD56^{bright}$) ratios between patients in active, and inactive stage Behçet's disease, and healthy controls (HCs) (1b).

and without comorbidity were included in our study. The clinical features of the cases are shown in Table 1. Mean age of 42 patients was 38 (19-60) (median, - (minimum-maximum)] [31 patients in the active phase with 37 (19-60) years, and 11 patients in the active phase with 38 (20-50) years]. None of the patients were receiving any medication (except two) and no infection was reported. Patients with more than one disease and infection were excluded due to the difficulty in evaluating disease activity. Fourteen volunteers with a mean age of 38 (20-54) years were included in the study as healthy controls.

Expression of $CD3^+$ T Lymphocytes and NK Cell Subsets

Expression of T cells and NK cell subsets in peripheral blood samples of patients and healthy subjects were evaluated by flow cytometry. The lymphocyte gating strategy in the SSC/FSC graph is shown in Figure 1a.

$CD3^+$ T cell ratio (p=0.002) was found significantly higher in lymphocyte population in patients with Behçet's disease compared to that of healthy subjects (Figure 1b, and Table 2). Similarly, $CD3^+$ T cell ratios of both active and inactive patients (p=0.01 and p=0.0009, respectively) were found to be significantly higher than those of healthy controls. There was no difference in $CD3^-CD16^+CD56^{dim}$ and $CD3^-CD16^-CD56^{bright}$ NK cell percentages of Behçet's patient groups and healthy subjects [p values for $CD3^-CD16^+CD56^{dim}$: p>0.11 (Behçet's), p>0.13 (active phase Behçet) and p>0.22 (inactive phase Behçet), and p values for $CD3^-CD16^+CD56^{bright}$: p>0.34 (Behçet's), p>0.31 (active phase Behçet) and p>0.57 (inactive phase Behçet)] (Figure 1b, Table 2).

RT-PCR Analyses of mRNA Results

There were no differences in *NKp30, NKp46,* and *NKG2D* mRNA expressions of patients with active and inactive Behçet's disease and healthy subjects [p values for *NKp30*: p>0.65, and for *NKp46*: p>0.20, and for NKG2D: p>0.49]. When the results were analyzed in active, and inactive patients, no significant difference was found between the two groups and healthy subjects [p values for perforin: p>0.69 (Behçet's), p>0.37 (active phase Behçet) and p>0.60 (inactive phase Behçet), and p values for granzyme: p>0.80 (Behçet's), p>0.74 (active phase Behçet) and p>0.86 (inactive phase Behçet)] (Figure 2).

When *perforin* and *granzyme* mRNA expressions were analyzed, no significant difference was found between patients in active or inactive phase of Behçet's disease and healthy controls (p>0.05). When the relationship between $CD3^+$ T lymphocyte, $CD3^-CD16^+CD56^{dim}$ and $CD3^-CD16^-CD56^{bright}$ NK cell ratios and mRNA levels

Table 1. Clinical and demographic characteristics of enrolled subjects.

	Patients with Behçet's Disease	Active phase	Inactive phase
n (M/F)	42 (22/20)	31 (16/15)	11 (6/5)
Age (years)	38 (19-60)	37 (19-60)	38 (20-50)
Leukocytes (x10^9/L)	8.6 (4.3-10.8)	9.3 (5.5-10.8)	8.6 (4.3-8.9)
Lymphocytes (x10^9/L)	2.7 (0.7-5.0)	2.8 (0.7-5.0)	2.5 (1.2-2.8)
HLA-B51 (P/N)	7/2	6/0	1/2
Infection	None	None	None

Table 2. Median (min-max) values of NK cell subgroups and $CD3^+$ T cell group in Behçet's patients and healthy controls.

	Patients with Behçet's Disease %Med. (min - max)	Patients in Active Phase of Behçet's Disease %Med. (min - max)	Patients in Inactive Phase of Behçet's Disease %Med. (min - max)	Healthy Subjects %Med. (min - max)
$CD3^+$	73.9 (54.4-81.8)	72.8 (54.4-81.8)	74.4 (69.2-81.4)	65.3 (57.0-78.3)
$CD3^-CD16^+CD56^{dim}$	6.9 (1.3-62.1)	6.8 (1.3-22.2)	8.4 (1.8-62.1)	8.2 (5.2-14.2)
$CD3^-CD16^-CD56^{bright}$	0.6 (0.1-1.5)	0.6 (0.1-1.5)	0.6 (0.1-1.0)	0.6 (0.2-1.6)

Med.: Median, Min.: Minimum, Max.: Maximum

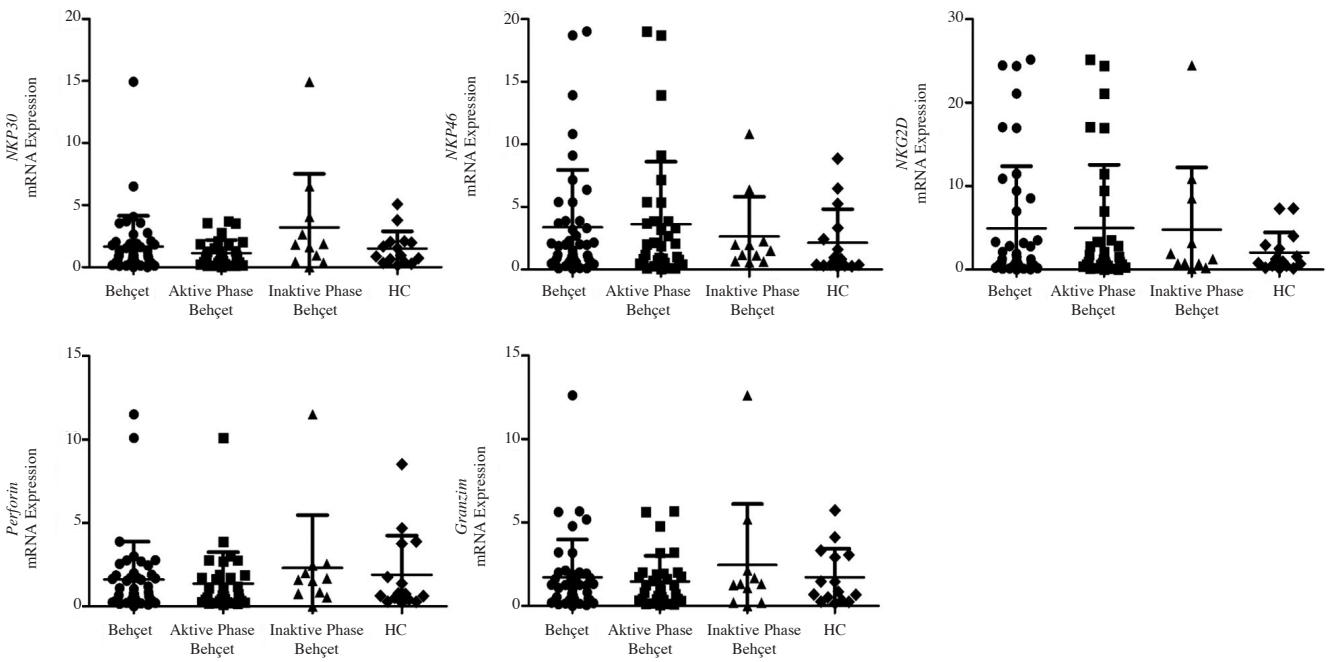

Figure 2. Comparison of $2^{-\Delta\Delta CT}$ values of NKp30, NKp46, NKG2D, perforin and granzyme mRNA expressions among Behçet's patients, active-inactive phase Behçet's patients, and healthy subjects (HC)

were evaluated by correlation analysis; a positive correlation (p=0.05, R=0.342) was observed between NKG2D mRNA level and $CD3^-CD16^+CD56^{bright}$ NK cell ratios in patients in the active phase of Behçet's disease. In addition, a positive correlation was found between $CD3^-CD16^+CD56^{dim}$ NK cell ratios and *NKG2D* (p=0.05, R=0.342) and *NKp46* mRNA (p=0.003, R=0.521) levels in patients with active Behçet's disease Also, positive correlation was found between *NKp46* mRNA levels and $CD3^+$ T cell ratio in patients with inactive Behçet's disease (p=0.04, R=0.623).

Discussion

Many studies have focused on investigating the immunological and genetic relationship of Behçet's disease, but the pathogenesis of the disease is still not clear[3]. The relationship between HLA-B51 and Behçet's Disease is the strongest data indicating that genetic factors are involved in the pathogenesis of the disease.[4,14] Immune cells such as T lymphocytes, NK and NKT cells may have a role in the pathogenesis of Behçet's disease.[15]

NK cells secrete various cytokines and also affect the acquired immune response. There are activator and inhibitory receptors on the NK cell surface. Immunoglobulin-like receptors (KIR) and NKG2A receptors are inhibitory receptors, and when they are triggered, NK cells cannot perform their function and lysis of target cells is inhibited.[9] In contrast, signals from the activator receptors stimulate NK cell functions.[16,17] It is claimed that they may play a role in the pathogenesis of autoimmune diseases by affecting the acquired immune response.

A decrease in NK cell numbers and functional impairment have been shown in rheumatoid arthritis (RA), type 1 diabetes, systemic lupus erythematosus (SLE), multiple sclerosis (MS).[18] The findings showing that NK cell activities and numbers are correlated with disease development and remission status in MS-SLE, suggest that NK cells have a role in the pathogenesis of the disease.[19] Studies have shown that NK cells play a role in the pathogenesis of autoimmune diseases such as type 1 diabetes.[4,20,21] It has been demonstrated that *NKp46* null mice develop resistance to inducing type-1 diabetes by streptozocin. Also, the development of diabetes is restricted in mice whose *NKp46* protein is blocked with antibodies.[9,22] It has been shown that the NK cell ratio and *NKp30* expression were increased in the chronic autoimmune disease as Sjogren's syndrome.[23] Although the numbers of NK cells have been shown to increase in Behçet's disease patients, no change has been found in the proportions of cells expressing activator receptors.[24] The results of these studies have demonstrated that the number of NK cells (expressing the NK receptor) do not provide the clear information about NK receptor expression levels. Therefore, in our study, total RNAs were isolated from the whole blood of patients, and NK receptor mRNA expression in active and inactive Behçet's disease were analyzed by qRT-PCR. It was observed that *NKp30, NKp46,* and *NKG2D* mRNA levels were increased-though not statistically significantly- in Behçet's disease patients compared to healthy controls. These activator NK receptors can increase the cytotoxic function of NK cells. Similarly, it was observed that *perforin*, and *granzyme* mRNA levels did not change in patients with Behçet's disease.

While NK cell receptors such as *NKp30* and *NKp46* are expressed at low levels in $CD8^+$ T and NKT cells; *perforin* and *granzyme* are expressed in $CD8^+$ T cells. In this study, mRNA levels were assessed in white blood cells. Studying NK receptors, perforin, and granzyme mRNA levels after NK cell isolation would have been more targeted. Our findings are consistent with other flow cytometry studies on NK receptors, *perforin*, and *granzyme* levels in patients with Behçet's disease.[22] Although it was not statistically significantly different, the elevated in *NKG2D* and *NKp46* mRNA levels were found to be correlated with the elevated number of $CD3^-CD16^+CD56^{dim}$ NK cells in patients in the active phase of Behçet's disease.

In conclusion, *NKp30, NKp46, NKG2D, perforin,* and granzyme mRNA expressions in peripheral blood mononuclear cells might not associated with the pathogenesis and inflammatory status of the disease. To define the role of NK cell activity in the pathogenesis of Behçet's disease, these data need to be validated with further functional studies.

Acknowledgments

The authors thank all the patients for participating in the study and thank Vuslat YILMAZ, Assoc. Dr. of Istanbul University-Aziz Sancar Institute of Experimental Medicine-Istanbul/Turkey, for her assistance in statistical analysis.

Ethics Committee Approval: Consent form was signed by all patients and healthy subjects (Istanbul University Faculty of Medicine Clinical Research Ethics Committee).

Conflict of Interest: The authors declare no conflict of interest to disclose.

Funding: This work was supported by Istanbul University BAP Project number: TDP2018-30155.

References

1. Azizlerli G, Kose AA, Sarica R, Gul A, Tutkun IT, Kulac M, et al. Prevalence of Behçet's disease in Istanbul, Turkey. Int J Dermatol. 2003;42(10):803-6. **[CrossRef]**
2. Gul A. Genetics of Behçet's disease: lessons learned from genomewide association studies. Curr Opin Rheumatol. 2014;26(1):56-63. **[CrossRef]**
3. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. Nat Genet. 2010;42(8):698-702. **[CrossRef]**
4. Gelmez MY, Cinar S, Aktas Cetin E, Ozcit-Gurel G, Babuna-Kobaner G, Erdugan M, et al. Inflammatory status might direct ILC and NK cells to IL-17 expressing ILC3 and NK subsets in Behçet's disease. Immunol Lett, 2021;235:1-8. **[CrossRef]**

5. McGonagle D, Aydin SZ, Gul A, Mahr A, Direskeneli H. 'MHC-I-opathy'-unified concept for spondyloarthritis and Behçet disease. Nat Rev Rheumatol. 2015;11(12):731-40. **[CrossRef]**

6. Carlsten M, Jaras M. Natural Killer Cells in Myeloid Malignancies: Immune Surveillance, NK Cell Dysfunction, and Pharmacological Opportunities to Bolster the Endogenous NK Cells. Front Immunol. 2019;10:2357. **[CrossRef]**

7. Zhang C, Hu Y, Shi C. Targeting Natural Killer Cells for Tumor Immunotherapy. Front Immunol. 2020;11:60. **[CrossRef]**

8. Aktas E, Erten G, Kucuksezer UC, Deniz G. Natural killer cells: versatile roles in autoimmune and infectious diseases. Expert Rev Clin Immunol. 2009;5(4):405-20. **[CrossRef]**

9. Barrow AD, Martin CJ, Colonna M. The natural cytotoxicity receptors in health and disease. Front Immunol. 2019;10: 909. **[CrossRef]**

10. Blunt MD, Khakoo SI. Activating killer cell immunoglobulin-like receptors: Detection, function and therapeutic use. Int J Immunogenet. 2020;47(1):1-12. **[CrossRef]**

11. Liu H, Wang S, Xin J, Wang J, Yao C, Zhang Z. Role of NKG2D and its ligands in cancer immunotherapy. Am J Cancer Res. 2019;9(10):2064-78.

12. Spits H, Bernink JH, Lanier L. NK cells and type 1 innate lymphoid cells: partners in host defense. Nat Immunol. 2016;17(7):758-64. **[CrossRef]**

13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. **[CrossRef]**

14. Giza M, Koftori D, Chen L, Bowness P. Is Behçet's disease a 'class 1-opathy'? The role of HLA-B*51 in the pathogenesis of Behçet's disease. Clin Exp Immunol. 2018;191(1):11-8. **[CrossRef]**

15. Zeidan MJ, Saadoun D, Garrido M, Klatzmann D, Six A, Cacoub P. Behçet's disease physiopathology: a contemporary review. Auto Immun Highlights. 2016;7(1):4. **[CrossRef]**

16. Di Vito C, Mikulak J, Mavilio D. On the way to become a natural killer cell. Front Immunol. 2019;10:1812. **[CrossRef]**

17. Marcus A, Gowen BG, Thompson TW, Iannello A, Ardolino M, Deng W, et al. Recognition of tumors by the innate immune system and natural killer cells. Adv Immunol. 2014;122:91-128. **[CrossRef]**

18. Tahrali I, Kucuksezer UC, Altintas A, Uygunoglu U, Akdeniz N, Aktas-Cetin E, et al. Dysfunction of CD3(-)CD16(+) CD56(dim) and CD3(-)CD16(-)CD56(bright) NK cell subsets in RR-MS patients. Clin Immunol. 2018;193:88-97. **[CrossRef]**

19. Tahrali I, Kucuksezer UC, Akdeniz N, Altintas A, Uygunoglu U, Aktas-Cetin E, et al. CD3(-)CD56(+) NK cells display an inflammatory profile in RR-MS patients. Immunol Lett. 2019;216:63-9. **[CrossRef]**

20. Traherne JA, Jiang W, Valdes AM, Hollenbach JA, Jayaraman J, Lane JA, et al. KIR haplotypes are associated with late-onset type 1 diabetes in European-American families. Genes Immun. 2016;17(1):8-12. **[CrossRef]**

21. Wang Y, Yuan W, Guo H, Jiang Y. High frequency of activated NKp46(+) natural killer cells in patients with new diagnosed of latent autoimmune diabetes in adults. Autoimmunity. 2015;48(4):267-73. **[CrossRef]**

22. Gur C, Porgador A, Elboim M, Gazit R, Mizrahi S, Stern-Ginossar N, et al. The activating receptor NKp46 is essential for the development of type 1 diabetes. Nat Immunol. 2010;11(2):121-8.

23. Rusakiewicz S, Nocturne G, Lazure T, Semeraro M, Flament C, Caillat-Zucman S, et al. NCR3/NKp30 contributes to pathogenesis in primary Sjogren's syndrome. Sci Transl Med. 2013;5(195):195ra96. **[CrossRef]**

24. Cosan F, Aktas Cetin E, Akdeniz N, Emrence Z, Cefle A, Deniz G. Natural killer cell subsets and their functional activity in Behçet's disease. Immunol Invest. 2017;46(4):419-32. **[CrossRef]**