Clinical Rheumatology (2019) 38:495–502
https://doi.org/10.1007/s10067-018-4288-7

ORIGINAL ARTICLE

Serum interleukin-37 level and interleukin-37 gene polymorphism in patients with Behçet disease

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Received: 12 July 2018 / Accepted: 5 September 2018 / Published online: 18 September 2018

Abstract

Behçet's disease (BD) is a chronic inflammatory disease. The etiopathogenesis of BD is not well understood and several cytokines and genetic factors have been investigated. Interleukin (IL)-37, which a member of IL-1 family is an anti-inflammatory cytokine. The aim of the study was to analyze serum IL-37 level and IL-37 gene polymorphisms to assess its possible role in BD. Two hundred twenty-three patients with BD and 80 healthy controls (HC) were enrolled. Serum IL-37 level was measured using an enzyme-linked immunosorbent assay (ELISA). Deoksiribo Nucleic acids (DNA) were extracted using a genomic DNA isolation kit. Single nucleotide polymorphism (SNP) of IL-37 gene (rs3811047) was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR/RFLP) methods. Serum IL-37 level was not significantly different in BD and HC ($p > 0.05$). Serum IL-37 level was not associated with the disease activity ($p > 0.05$). However, its level was higher in mucocutaneous involvement compared with systemic involvement ($p = 0.002$) and HC ($p = 0.005$). IL-37 gene polymorphisms were similar in BD and HC ($p > 0.05$). IL-37 may play a role in the etiopathogenesis of BD by contributing to manifestation with more moderate clinical symptoms.

Keywords Behçet's disease · Cytokine · Gene polymorphism · Interleukin-37

Introduction

Behçet's disease (BD) is a chronic systemic inflammatory disease characterized by recurrent oral aphthous and genital ulcers and numerous systemic manifestations such as cutaneous, ocular, neurologic, vascular, articular, and gastrointestinal involvement. Although the etiopathogenesis of BD is not well understood, microbial agent triggers, environmental factors, genetic predisposition, endothelial cell dysfunction, neutrophil hyperfunction, and autoimmune mechanisms have been supposed [1, 2]. BD is more common in the countries extending form Mediterranean countries to the Far East. It is confirmed that interactions among the genetic and environmental factors play an essential role in the pathogenesis of BD [3]. In this context, various cytokines and gene polymorphisms of cytokines were shown to be involved in BD's pathogenesis [1]. Recently, some studies were published to point out the possible role of IL-37 in the pathogenesis of BD [1, 4].

Interleukin-37 (IL-37), a member of the interleukin-1 (IL-1) family, is recently identified as a natural suppressor of innate inflammation and acquired immunity [5]. The locus containing genes for IL-37 is located on chromosome 2 [6]. IL-37 is expressed in various normal cells and tissues such as natural killer (NK) cells, stimulated B cells, monocytes, skin keratinocytes, epithelial cells, lymph node, thymus, lung, colon, uterus, and bone marrow [7]. There are five different splice variants of IL-37 termed IL-37 a–e. Among them, IL-37b is the largest and best-characterized isoform [5]. Mechanisms of the biological functions of IL-37 seems to depend on interaction with IL-18 receptor α (IL-18Rα) and the orphan decoy IL-1R8 for suppressing the production of cytokines, levels of transcription factors, and activation of

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signaling kinases. It was shown that IL-37 has complex biological functions in different diseases [5]. The role of IL-37 in the pathogenesis of BD is still unknown.

Single nucleotide polymorphism (SNP) rs3811047 G/A polymorphism are one of the IL-37 genes variant [8]. SNP is the DNA sequence polymorphism caused by single nucleotide (A, G, C, T) variation. It is called the new generation genetic marker and thought that, determination of genotype distribution of SNP can reveal to the relation between the certain genotype and disease susceptibility [9]. Some studies showed that SNP rs3811047 is related to autoimmune diseases [10]. However, clinical significance of various gene polymorphisms such as human leukocyte antigen (HLA) genes and non-HLA genes were investigated in BD; to our knowledge, there is one study about IL-37 gene polymorphism in BD [11]. The aim of this study is to determine the significance of IL-37 pathway on the pathogenesis of BD by measuring the serum level of IL-37 and analyzing IL-37 gene polymorphisms in Turkish patients with BD.

Materials and methods

Participants

Two hundred twenty-three patients with BD admitted to Ankara University Faculty of Medicine, multidisciplinary BD diagnosis and treatment unit between 2012 and 2013 were included in this study. Eighty healthy persons without history of any inflammatory and autoimmune diseases were selected as a control group (HC). All patients who met the 1990 international criteria for the classification of BD were recruited into this study [12]. Being younger than 16 years old, having a concomitant disease and pregnancy were exclusion criteria. The study was approved by the Ethics Committee of Ankara University and all participants signed an informed consent prior to the initiation of the study. Patients were further subgrouped according to clinical manifestations as mucocutaneous or systemic. The clinical activity score was determined by the Behçet Syndrome Activity Scale (BSAS) which based on patient report [13]. BSAS consists of ten questions and is scored 0–10 each for a total score of 0–100. It asks about various symptoms over previous months before the current clinic visit including visual analogue scales (VAS) for patient's level of discomfort with regard to oral ulcers, genital ulcers, skin lesions, and current disease activity. Others are the number of oral ulcers, genital ulcers, and skin lesions and presence of gastrointestinal involvement, systemic involvement, or eye involvement. We used its validated Turkish version for our study [14]. BD patients were considered as active based on the opinion of a rheumatologist during the evaluation of patients and BSAS ≥ 10. All the patients and controls were unrelated to each other and collected mainly from the central part of Anatolia, Turkey.

Laboratory analysis

All patients with BD underwent routine laboratory assessment including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Serum samples for IL-37 measurement were obtained from all subjects by centrifugation of venous blood and stored at − 80 °C until analysis; samples for IL-37 gene polymorphisms are stored at − 20 °C after DNA isolation. The serum level of IL-37 (MBS70512, MyBioSource, USA) were measured by ELISA. Absorbance readings were carried out on the Rayto microplate reader. Concentrations were determined from the curve obtained with the standards.

Genotype analysis

DNA was extracted from whole blood using a genomic DNA isolation kit (QIAGEN Gentrapurgene). Analysis of the IL37 gene (rs3811047) A/G polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR/RFLP) methods. In this SNP, adenines changed to guanine, resulting in an amino acid change from threonine to alanine at codon 42 (p.Thr42Ala).

Following primers were designed by using primer-3 program [15, 16]; IL37 F 5'-AATGTCATTACTATCATGCT GCTC and IL37 R5'-TTCTACAATTCTCCCACCCTGT were used in the study. PCR was performed using 25 ng genomic DNA, one unit Taq DNA polymerase (Fermentas, Lithuvania), a total of 20 pmol of each primer, and 5 nmol deoksi NTPs under the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation (94 °C, 50 s), annealing (59 °C, 50 s), extension (72 °C, 50 s), and a final extension at 72 °C for 7 min. Ten microliters of the amplified product were subjected to restriction enzyme digestion with AciI (New England Biolabs USA). Digestion results were visualized on 2% agarose gel under UV light.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software program version 11.5. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not normally distributed. Descriptive analyses were presented using medians and minimum-maximum (min-max) for the non-normally distributed. Nominal values were expressed as number (*n*) and percentage (%). As IL-37 levels were not normally distributed, the Kruskal-Wallis tests

were conducted to compare the parameters. The Mann-Whitney U test was performed to test the significance of pairwise differences using post hoc correction to adjust for multiple comparisons. ROC curve analysis was used to test the hallmark of IL-37 in predicting BD subgroups. When a significant cutoff value was observed, the sensitivity, specificity, positive, and negative predictive values were presented. Binary logistic regression models were used to analyze the SNP data. Results were expressed as odds ratios (OR) with corresponding 95% confidence intervals (95% CI). A p value of less than 0.05 was considered to show a statistically significant result and all significant levels were two-tailed.

Results

The demographics

Ninety-nine (44.4%) of BD patients were male and 124 (55.6%) were female. Mean age was 38.4 ± 11.5 years for BD patients. Median BSAS value of BD patients was 13 (1–65) (Table 1). Ninety-two (41.3%) patients had mucocutaneous involvement while 131 (58.7%) patients had systemic involvement. One hundred forty-two (63.7%) of 223 patients were considered as having active disease (Table 2).

Serum IL-37 level

Median serum IL-37 level was 10.0 pg/ml in patients with BD and 7.8 pg/ml in HC group which was statistically similar (p = 0.118). Median IL-37 level in patients with mucocutaneous involvement and systemic involvement were 11.1 (7.8–146.2) pg/ml and 8.6 (7.8–701.9) pg/ml respectively. Median IL-37 level was significantly higher in patients with mucocutaneous involvement compared to patients with systemic involvement (p = 0.002) and HC (p = 0005) while the median IL-37 level in patients with systemic involvement was similar to HC (p = 0.773) (Table 2). Serum IL-37 level was similar both in patients with active and inactive disease (median 9.9 and 9.3 respectively; p = 0.645) (Table 2). In inactive patients with BD, serum level of IL-37 of patients with mucocutaneous involvement was significantly higher than patients with systemic involvement (median 13.0 and 7.8 respectively, p = 0.005). In active patients with BD, serum IL-37 level in patients with mucocutaneous involvement was higher from systemic involvement that was slightly significant (median 10.8 and 8.8 respectively, p = 0.057). There was no

Table 1 Demographic and clinical characteristics of Behçet's disease patients (n = 223)

	Behçet's disease	Healthy controls	p value
Age (mean ± SD)	38.41 ± 11.45	36.72 ± 10.06	$p > 0.05$
Gender (male/female), n (%)	99 (44.4)/124 (55.6)	40 (50)/40 (50)	$p > 0.05$
Disease duration (year) (mean ± SD)	9.34 ± 8.55	–	
Clinical manifestations, n (%)			
Oral ulcer	206 (92.4)		
Genital ulcer	171 (76.7)	–	
Papulopustular erythema	133 (59.6)	–	
Pathergia	103 (58.2)	–	
Erythema nodosum	77 (34.5)	–	
Arthralgia	121 (54.3)	–	
Arthritis	22 (9.9)	–	
Thrombosis	48 (21.5)	–	
CNS involvement	32 (14.4)	–	
GIS involvement	23 (10.4)	–	
Uveitis	86 (38.6)	–	
Ongoing medications, n (%)	218(97.8)		
Colchicine	192(86.1)		
Azathioprine	59(26.5)		
Corticosteroids	31(13.9)		
Cyclosporine	4(2)		
Cyclophosphamide	4(2)		
Sulphasalazine	2(1)		
BSAS median (min-max)	13 (1–65)	–	

SD standard deviation, *CNS* central nervous system, *GIS* gastrointestinal system

Table 2 Comparison of plasma IL-37 levels in each group*

	n (%)	Age (year) mean ± SD	Gender (F/M)	BSAS median (min-max)	IL-37 median (min-max)
Inactive cases[a]	81 (36.3)	40.8 ± 11.8	43/38	4.0 (1–9)	9.3 (7.8–146.3)
Active cases[b]	142 (63.7)	37.0 ± 11.1	81/61	18 (10–65)	10.1 (7.8–701.9)
Mucocutaneous involvement[c]	92 (41.3)	38.7 ± 12.2	66/26	12.5 (1–57)	11.0 (7.8–146.3)
Systemic involvement[d]	131 (58.7)	38.2 ± 10.9	58/73	14 (1–65)	8.5 (7.8–701.9)
Healthy controls[e]	80 (26.4)	36.7 ± 10.1	40/40	–	7.8 (7.8–54.8)

* Mann-Whitney U test with Bonferroni correction, multiple comparisons were done by using Kruskal-Wallis test. $p = 0.263$ for IL-37 in comparisons of [a, b, e]

$p = 0{,}003$ for IL-37 in comparisons of [c, d, e]

$p = 0.002$ for IL-37 in comparisons of [c, d]

$p = 0.005$ for IL-37 in comparisons of [c, e]

$p = 0.773$ for IL-37 in comparisons of [d, e]

significant association in patients with mucocutaneous involvement between serum IL-37 level and active or inactive disease (median 10.8 and 13.0 respectively, $p = 0.594$). Similarly, there was no significant association in patients with systemic involvement between serum IL-37 level and active or inactive disease (median 7.8 and 8.8 respectively, $p = 0.248$). Besides disease activity score, there were no any correlations between serum IL-37 level and ESR, CRP. There was no any association between treatment and IL-37 ($p > 0.05$).

The association of serum IL-37 level with clinical parameters of BD patients was analyzed and results showed that mucocutaneous manifestations except PPE also joint involvement (arthralgia) were positively associated with serum IL-37 level (Table 3).

Clinicopathological significance of IL-37

The level of IL-37 in determining the difference between the HC with mucocutaneous involvement (area under the curve, 0.62; $p = 0.006$) and the mucocutaneous involvement and systemic involvement (area under the curve: 0.62; $p = 0.003$) is distinctive. The cutoff value of IL-37 was 7.9 pg/ml by the ROC curve with a sensitivity 76.1% and specificity 52.5% between mucocutaneous involvement and HC groups. The cutoff value of IL-37 was 9.9 pg/ml by the ROC curve with sensitivity 65.2% and specificity 60.3% between mucocutaneous involvement and systemic involvement (Figs. 1 and 2). The level was not distinctive in determining the difference between systemic involvement and HC (area under the curve, 0.51; $p = 0.785$).

Further, we divided our patients into two groups; IL-37 < 9.9 as a low level of IL-37 and IL-37 ≥ 9.9 as a high level of IL-37. One-hundred fourteen (51.1%) patients showed a low level of IL-37. Sixty-one percent of these patients presented as systemic manifestations. In this group, only 37% of patients presented mucocutaneous manifestations. Differences in clinic spectrum between these groups were shown in Table 4.

Table 3 Comparison of serum IL-37 levels in BD patients according to their clinical parameters

	n (%)	Median IL-37 level (pg/ml) min-max	*p* value*
Oral ulcer	206(92.4)	10.23 (7.81–701.90)	0.005*
Genital ulcer	171(76.7)	10.34 (7.81–701.90)	0.009*
Pathergia	103(58.2)	10.30 (7.81–146.28)	0.015*
Papulopustular erythema	133 (59.6)	10.23 (7.81–701.90)	0.304
Erythema nodosum	77 (35.0)	10.75 (7.81–517.72)	0.004*
Arthralgia	121(54.3)	10.68 (7.81–701.90)	0.022*
Arthritis	22(9.9)	10.29(7.81–186.30)	0.665
Thrombosis	48(21.5)	7.81 (7.81–186.30)	0.079
CNS involvement	32(14.4)	7.81 (7.81–44.20)	0.060
GIS involvement	23(10.4)	7.81 (7.81–60.55)	0.064

CNS central nervous system, *GIS* gastrointestinal system

Serum IL-37 level was compared between patients' each clinical signs whether did or did not have

*$p < 0.05$ statistical significant

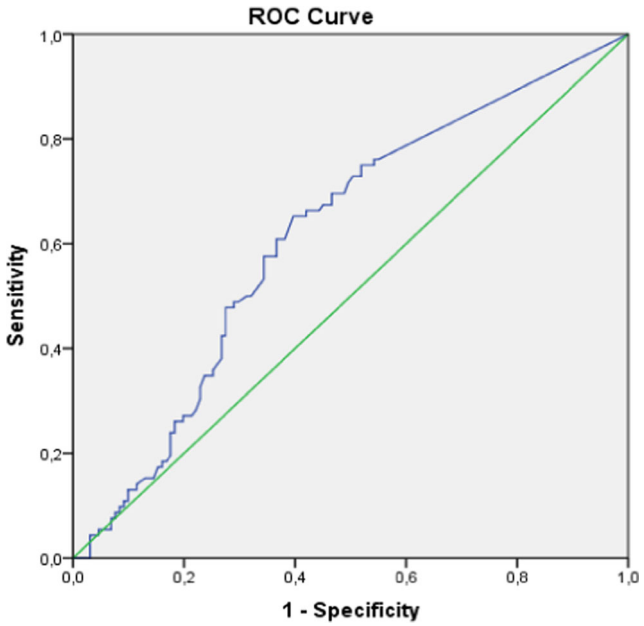

Fig. 1 Serum IL-37 levels between mucocutaneous involvement and HC groups and determination of cutoff point

IL-37 gene polymorphisms

There was no significant difference in terms of the genotypic and allelic distributions of rs3811047 G/A polymorphisms between BD and HC groups (Table 5). Also, there was no significant association between IL-37 gene polymorphism and each clinical findings of BD ($p > 0.05$ for oral ulcer, genital ulcer, pathergia, papulopustular erythema, erythema nodosum, arthralgia, uveitis, GIS and SSS involvement, arthritis, and thrombosis).

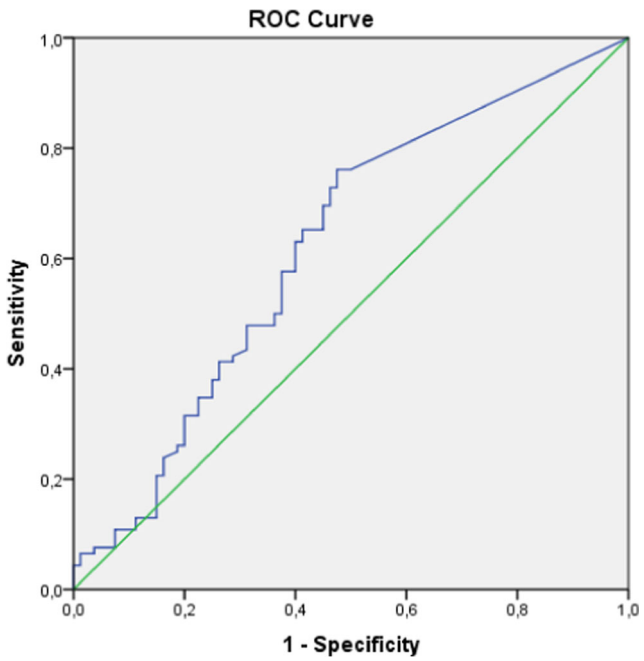

Fig. 2 Serum IL-37 levels between mucocutaneous involvement and systemic involvement and determination of cutoff point

Discussion

In this cross-sectional study, serum IL-37 level and IL-37 gene polymorphisms were evaluated in Turkish patients with BD. Serum IL-37 level was similar in BD and HC groups. However, its level was higher in patients with mucocutaneous involvement compared to patients with the systemic involvement and HC. It indicated that IL-37 level was positively associated with mucocutaneous manifestations. IL-37 gene rs3811047 G/A polymorphisms were similar in each group. Any significant differences could be found regarding serum IL-37 level and IL-37 gene polymorphisms between active and inactive patients. To our knowledge, this is the first study to evaluate the possible role of IL-37 in BD from our country. Also, our study can be accepted as the first detailed study which evaluates the association of IL-37 with all manifestations of clinical symptoms.

The IL-1 family cytokines has been well characterized and plays an important role in regulating the immune response. Whereas, most of the family members are pro-inflammatory; IL-37, a newly discovered cytokine of this family, is taking attention as being anti-inflammatory. IL-37 plays a role in innate immunity by limiting production of inflammatory cytokines as well as in acquired immunity by augmenting T-regulatory (T-reg) cells and inhibiting activation of dendritic cells maturation [5, 17]. There are few studies about the role of IL-37 in BD. One of them which presented as the first study in this issue by Ye et al. reported that decreased IL-37 expression may be involved in the pathogenesis. Their results showed that IL-37 mRNA levels and protein expression in PBMCs were decreased in active BD as compared to HC. They demonstrated that dendritic cells (DCs) stimulated with recombinant IL-37 (rIL-37) showed a decreased expression of pro-inflammatory cytokines as IL-6, IL-1β, and TNF-α. Also, rIL-37 significantly inhibited the production of reactive oxygen species in DCs. Recombinant IL-37-treated DCs inhibited Th1 and Th17 cell responses that have an important role in BD [18]. Another study found a negative correlation between IL-37 expression in BD patients and increased inflammatory response. They reported a decreased IL-37 expression in active BD patients. Corticosteroid treatment in active BD patients leads to increased expression of IL-37 mRNA. They suggested that the immunosuppressive effect of corticosteroids can be explained by up-regulating IL-37 production and reducing inflammatory cytokines. They did not find any correlation between IL-37 mRNA or IL-37 protein level and clinical manifestations [1]. In our study, serum IL-37 level was higher than HC in BD patients but the difference did not reach the statistical significance. Nevertheless, increasing level of

Table 4 Differences in clinical manifestations according to serum IL-37 level

n (%)	Serum IL-37 level < 9.9	Serum IL-37 ≥ 9.9	*p* value*
Mucocutaneous involvement	34 (37.0)	58 (63.0)	< 0.001*
Systemic involvement	80 (61.1)	51 (38.9)	< 0.001*
Oral ulcer	101 (49.0)	105 (51.0)	0.03*
Genital ulcer	80 (46.8)	91 (53.2)	0.019*
Pathergia	48 (46.6)	55 (53.4)	0.016*
Papulopustular erythema	65 (48.9)	68 (51.1)	0.414
Erythema nodosum	32 (41.6)	45 (58.4)	0.042*
Arthralgia	53 (43.8)	68 (56.2)	0.017*
Arthritis	11 (50.0)	11 (50.0)	0.929
Thrombosis	30 (62.5)	18 (37.5)	0.075
CNS involvement	19 (59.4)	13 (40.6)	0.300
GIS involvement	15 (65.2)	8 (34.8)	0.147

CNS central nervous system, *GIS* gastrointestinal system

*$p < 0.05$ statistical significant

IL-37 is thought as an excessive inflammatory response in BD. In contrast to these studies, serum IL-37 level did not show significant differences between subgroups respecting disease activity. Our discordant results with these previous studies may arise from analyzing only serum level of IL-37, not the expression of IL-37 in the cultured PBMC. It may also be that we could not measure the level of serum IL-37 in the active patients when they become inactive. Also, the differences may arise from the numbers of study populations. But we found lower levels of IL-37 in active patients when we looked mucocutaneous or systemic separately. In our opinion IL-37 has an anti-inflammatory effect at tissue level, but its quantitative value must be interpreted as a relative marker according to patients' clinic conditions. Further studies will clear up the significance of IL-37 serum level on this issue.

An important aspect of our study was that we found significant association between the level of IL-37 and mucocutaneous manifestations. Additionally, most patients with low level of IL-37 had systemic involvement. This can be interpreted that high IL-37 has a protective effect in BD patients with mucocutaneous involvement by inhibiting more severe clinical findings. It is interesting that serum IL-37 level was associated with mucocutaneous manifestations of BD in both active and inactive patients but not for systemic involvement. Contrarily, we could not find any significant association between serum IL-37 level and disease activity in the mucocutaneous or systemic involvement. It can be thought that IL-37 may play an important role in clinical manifestations compared to disease activity. Mechanism of action of IL-37 on clinical signs remains unclear. Boraschi et al. suggested that Smad3, a protein necessary for suppression of TGF β, is a key in IL-37-mediated inhibition of inflammatory cytokine production [19]. The lack of intact TGF-β signaling via Smad3 results in an increased pro-inflammatory response in the skin [20]. High level of IL-37 in mucocutaneous manifestations that we found in our results supports that IL-37/Smad3 association may be damaged in these patients. Another study reported that IL-37 acts as a suppressor of thymic stromal lymphopoietin (TLSP). The expression of TSLP increased in BD patients' cutaneous lesions and is responsible for impaired skin integrity [2]. Similar with our results, Chi et al. reported that arthralgia was associated with IL-37 in patients with Still's disease [21]. The pathophysiology of arthralgia in these patients is still not clear; they declared that Treg, NK, and Th17 cells may play a role. IL-37 acts as a major mediator of NK cell suppression [22]. We concluded that different clinical manifestations may arise from mutually induced pathways and/or different pathways related to IL-37. Understanding the anti-inflammatory effect of IL-37 on the clinical manifestations with the support of further study results may be a pioneer to new treatment regimes.

First report about SNP rs3811047 associated with was AS published from Chinese population [10]. According to another study result [19], AG genotype was significantly lower than GG genotype in AS patients as compared to HC. In AG genotype,

Table 5 The association of IL-37 gene (rs3811047) polymorphism with Behçet diseases

	Behçet patients *n* (%)	Healthy controls *n* (%)	*p**	OR (95% Cl)
Genotype				
GG	102 (45.7)	40 (50)	Reference	
AG	25 (11.2)	15 (18.8)	0.258	0.65 (0.31–1.36)
AA	96 (43.1)	25 (31.2)	0.161	1.50 (0.85–2.66)
Allele				
G	229 (51.3)	95 (59.4)	reference	
A	217 (48.7)	65 (40.6)	0.081	1.38 (0.96–1.99)

* p value for test of the association

ESR and CRP levels were lower than GG genotype [23]. Shi et al. revealed that there is no correlation between rs3811047 SNP and RA susceptibility. IL-37 gene was correlated with arthritis and daily living ability [8]. Recent studies revealed that there is an association between IL-37 gene polymorphisms (rs3811047) and autoimmune diseases such as psoriatic arthritis and autoimmune thyroid disease. Similar to the results of the study of Lin et al., the frequency of AG genotype was significantly lower, and the frequency of GG genotype was significantly higher in 23 patients of BD with uveitis as compared to HC. In this study, IL-1β and TNF-α levels were higher in GG genotypes than AG genotypes; IL-6 was slightly higher. GG genotype carriers of rs3811047 had increased mRNA expression of the gene and response of pro-inflammatory cytokine [11]. The minor allele A was associated with decreased expression level of IL-37 mRNA [17]. Conversely, we did not found any significant association between IL-37 gene polymorphisms (rs3811047) and neither BD nor clinical signs. This finding may result from geographical and ethnic differences. On the other hand, distinct IL-37 gene polymorphisms may play a role in Turkish patient population. Further studies about IL-37 gene polymorphisms (rs3811047) in Turkish patients may contribute our results.

However, there are some limitations in our study. First, we did not examine serum IL-37 level and IL-37 gene polymorphisms (rs3811047) in species extracted from tissue material. Second, we investigated only one SNP of IL-37 gene that the others (rs2723176 and rs67177710) [8] may contribute to the development of Turkish BD patients. Finally, it was a hospital-based prospective study, so patients were unrepresentative for the Turkish population or general population.

In conclusion, we did not demonstrate an association of IL-37 gene polymorphism with BD neither clinical findings nor disease activity. But, we demonstrated that IL-37 cytokine is significantly elevated in patients with mucocutaneous involvement and arthralgia. This study supports that IL-37 plays role in pathogenesis of BD and can be responsible from the clinical manifestations. IL-37 can be used as a diagnostic tool in the future, moreover it might have an efficacious therapeutic potential.

Funding information This study received financial assistance from the Ankara University Scientific Research Project Coordination Unit.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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