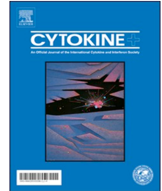
Cytokine 127 (2020) 154994

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine



# Interleukin-17 mRNA expression and serum levels in Behçet's disease

Golamreza Jadideslam[a,b,c,1], Houman Kahroba[b,d,2], Khalil Ansarin[b,1], Ebrahim Sakhinia[a,3], Alireza Abhar[d,2], Shahriar Alipour[e], Jafar Farhadi[a,3], Sam Seydi Shirvani[a,3], Masoud Nouri-Vaskeh[a,3], Saeed Mousavi[d,2], Alireza khabbazi[a,*,3]

[a] *Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Iran*
[b] *Department of Molecular Medicine, Tabriz University of Medical Sciences, Iran*
[c] *Faculty of Medicine, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[d] *Tabriz University of Medical Sciences, Iran*
[e] *Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

ARTICLE INFO

*Keywords:*
Behçet's disease
Interleukin-17
T-helper 17
Uveitis
Disease activity

ABSTRACT

Behçet's disease (BD) was considered a T-helper 1 (Th1)-mediated autoimmune disease, but with the introduction of Th17 cells, their role in the pathogenesis of BD was also addressed. Despite studies on IL-17 in BD, the prognostic value of this cytokine in BD is unclear. The aim of this study is to determine the IL-17 mRNA expression rate and serum levels in patients with BD and its correlation with clinical manifestations and activity of BD. Forty-six BD patients in the active phase of the disease and 70 healthy controls were recruited in this study. BD activity was measured by Behçet's disease current activity form (BDCAF), Iranian Behçet's disease dynamic activity measure (IBDDAM) and total inflammatory activity index (TIAI). The IL-17 mRNA expression and serum levels were significantly higher in the BD patients compared with the healthy controls. These parameters in the BD patients aged < 25 at disease onset, positive pathergy test, and positive HLA-B5 and HA-B51 were significantly higher than the healthy controls ($P < 0.05$). The IL-17 serum level in the patients with active uveitis was lower than the patients with in-active uveitis. There was no association between other clinical manifestations of BD and these parameters. No significant correlation was found between BDCAF and IBDDAM with IL-17 mRNA expression and serum levels. However, TIAI had a significant and negative correlation with the serum levels of IL-17.

## 1. Introduction

Behçet's disease (BD) is a systemic vasculitis characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, skin lesions and arthritis [1]. Although the pathogenesis of BD is not fully understood, environmental factors, genetic background, and epigenetic mechanisms are thought to play a key role in the aberrant activation of the immune system [2–5]. The role of innate and acquired immunity in the pathogenesis of BD has been demonstrated [2]. Although BD was considered a T-helper 1 (Th1)-mediated autoimmune disease, with the introduction of Th17 cells, their role in the pathogenesis of BD was also addressed [6]. Innate immune system by the overproduction of inflammatory mediators leads to an increase of Th1- and Th17-mediated cytokines, which play a prominent role in the pathogenesis of BD [2,7]. Decreased regulatory T cells (Treg) activity in BD patients plays a critical role in Th1/Th17 polarization [8]. During immune system activation, interleukin (IL)-6 and transforming growth factor β (TGF-β) cause differentiation of Th17 cells from naive T cells [9]. IL-26 is a pro-inflammatory cytokine, which has recently been reported to induce Th17 formation [10]. Th9 cells by producing IL-9, can induce Th17 formation during BD attacks [11]. Th17 cells by producing IL-17 and other pro-inflammatory cytokines like IL-1, IL-6, IL-8, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), can recruit immune effector cells and promote inflammation in inflammatory/autoimmune diseases [9].

Many studies over the past two decades have shown the importance of Th17 cells and IL-17 in the pathogenesis of inflammatory diseases such as rheumatoid arthritis (RA), inflammatory bowel disease,

* Corresponding author at: Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Golgasht St., 5166614756 Tabriz, Iran.
*E-mail addresses:* dr_khabbazi@yahoo.com, dr.khabazi@gmail.com (A. khabbazi).
[1] Molecular Medicine Department, Faculty of Modern Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
[2] Tabriz University of Medical Sciences, Tabriz, Iran.
[3] Connective Tissue Diseases Research Center, Flat 4, Emamreza Hospital, Tabriz, Iran.

https://doi.org/10.1016/j.cyto.2020.154994
Received 5 June 2019; Received in revised form 1 January 2020; Accepted 8 January 2020
1043-4666/ © 2020 Published by Elsevier Ltd.

systemic sclerosis, multiple sclerosis, psoriasis, ankylosing spondylitis (AS), uveitis and Vogt-Koyanagi-Harada disease [12–20]. Recently, the crucial role of the IL-17 axis has been shown in the pathogenesis of BD. Shen et al. studied IL-17 serum levels and production by peripheral blood mononuclear cells (PBMCs) in patients with BD compared with healthy controls [21]. They found higher serum IL-17 and IL-17 production by PBMCs in BD patients compared with healthy controls and in patients with active uveitis compared with those without. The high ratio of Th17/Th1 and Th17/Treg found in BD patients suggest that IL-17 over-expression may correlate with the clinical manifestations of BD [22–27].

Despite studies on IL-17 in BD, the prognostic value of this cytokine in BD is unclear. The aim of this study is to determine the IL-17 mRNA expression rate in PBMCs and serum levels in patients with BD in comparison with healthy controls and its correlation with clinical manifestations and BD activity.

## 2. Materials and methods

### 2.1. Studied groups

Forty-six BD patients with active disease from a cohort of BD in the Connective Tissue Diseases Research Center and 70 age- and sex -matched healthy controls were recruited in the study. BD was diagnosed based on the International Criteria for Behçet's Disease (ICBD) [28]. The participants were Azeri and were not related to each other. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethic code: TBZMED.REC.1395.1357) and the participants signed informed consents before enrolling in the study. The study was designed according to the Helsinki humanity research declaration (2008). BD activity was measured using the Behçet's Disease Current Activity Form (BDCAF), Iranian BD Dynamic Activity Measure (IBDDAM), and Total Inflammatory Activity Index (TIAI) [29–31]. BDCAF evaluates BD activity in all organs. IBDDAM measures BD activity in all organs except eyes and TIAI measures BD activity in eyes [29]. BDCAF ≥ 1 was considered as active disease [31]. TIAI ≥ 3.5 was considered as active uveitis [31]. The demographic and clinical characteristics of the participants are shown in Table 1.

### 2.2. RNA extraction and cDNA synthesis

We obtained 5 mL of peripheral blood prepared in the EDTA tubes. PBMCs were separated by Ficoll (Lymphodex, Inno-Train, Germany) density-gradient centrifugation (Sigma- Aldrich GmbH, Munich, Germany) and were stored at −80 °C until next step. Total RNA was extracted from the participants' blood samples by the protocol of TRIzol® (Invitrogen, San Diego, CA) on the basis of the manufacturer's instructions. The quality of RNA was checked by NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The extracted RNA was stored at −80 °C until complementary DNA (cDNA) synthesis (Thermo Fisher Scientific, Waltham, MA) was carried on.

**Table 1**
Demographic, clinical and laboratory characteristics of the participants.

| | Behçet's disease group (N = 46) | Healthy control group (N = 70) | P-value |
|---|---|---|---|
| Age (mean ± SD) years | 39.83 ± 11.1 | 37.10 ± 7.6 | 0.118 |
| Gender (male/female) | 29/17 (1.7) | 46/24 (1.9) | 0.659 |
| Oral aphthous ulcer (%) | 44 (95.7) | – | |
| Uveitis (%) | 35 (76.1) | – | |
| Genital ulcer (%) | 23 (50) | – | |
| Positive Pathergy test (%) | 14 (30.4) | – | |
| Pseudofolliculitis (%) | 11 (23.9) | – | |
| Erythema nodosum (%) | 9 (19.6) | – | |
| Arthritis (%) | 9 (19.6) | – | |
| Phlebitis (%) | 6 (13) | – | |
| Epididymitis (%) | 3 (6.5) | – | |
| CNS involvement (%) | 1 (2.2) | – | |
| HLA-B5 (%) | 27 (58.7) | – | |
| HLA-B51 (%) | 25 (54.3) | – | |

SD, standard deviation; CNS, central nervous system; HLA, human leukocyte antigen

### 2.3. IL-17 Real-time PCR

The IL-17 mRNA expression was evaluated in PBMCs by Real-time PCR on MIC instrument (BioMolecular Systems, AUSTRALIA). Quantitative Real-time PCR (qRT-PCR) was provided using specific primer sets (Eurofins Genomics) for IL-17 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference genes (see Table 2). 6.25 µL of Ampliqon SYBR Green master mix (RealQ Plus Master Mix Green high ROX™, Ampliqon, Copenhagen, Denmark) was used for q-PCR: 1 µL of cDNA, 0.5 µL of each IL-17 and GAPDH primers and 4.25 µL DW was added to reach the final volume of 12.5. Initial enzyme activation was performed at 95 °C for 15 min. 40 cycles of denaturation was performed for 20 s at 95 °C and annealing was applied for 30 s at 58.5 °C. IL-17 mRNA expression was normalized to GAPDH as internal reference with $2^{-\Delta\Delta Ct}$ formula [32]. All the reactions were performed at least in duplicate.

### 2.4. Quantification of IL-17 by enzyme-linked immunosorbent assays

The serum levels of IL-17 were detected using enzyme-linked immunosorbent assays (ELISA) in duplicates and according to the manufacturer's instructions (IBL ELISA kit™, IBL Inc., Hamburg, Germany). All samples were analyzed in the same batch. The absorbance and concentration of IL-17 were measured and calculated from a standard curve using a microplate reader Stat Fax 3200® Microplate Reader (USA).

### 2.5. Statistical analysis

Statistical analysis was performed using SPSS (SPSS Inc, Chicago, IL, USA) v 16. Detailed data were expressed as the mean ± SD and for out of range data by median. Normal distributions were analyzed by the Kolmogorov-Smirnov test with Lilliefors correction. The independent *t*-test and Mann–Whitney *U* test were used to make a comparison between the parametric and non-parametric variables. The Pearson rank correlation was used and $P < 0.05$ was considered to be statistically significant. The graphs were drawn using the GraphPad Prism v.6.00 (GraphPad Software, La Jolla, CA) and SPSS v. 16.

## 3. Results

In this study, we analyzed IL-17 mRNA expression and serum levels in 46 patients with active BD and 70 healthy controls. IL-17 mRNA expression in the BD and control groups were 2.83 (median = 2.79; min = 1.07, max = 6.02) and 1.99 (median = 0.68; min = 0.37, max = 9.42), respectively (Fig. 1). We also found a higher serum levels of IL-17 in the BD patients [4.24 (median = 3.98; min = 1.53, max = 6.92)] compared with the healthy controls [3.19 (median = 2.97; min = 1.04, max = 6.19) pg/mL]). A positive correlation was observed between IL-17 mRNA expression and serum levels ($r = 0.232$, $P = 0.012$) (Fig. 2). In addition, we analyzed the association of IL-17 mRNA expression and serum levels with demographic, clinical and laboratory features of the BD patients (Table 3). IL-17 mRNA expression and serum levels in the BD patients with younger age at disease onset, positive pathergy test and positive HLA-B5 and HA-B51 were higher. However, this figure did not reach to a significant level for IL-17 mRNA expression in the BD patients aged < 25 at disease presentation. Serum levels of IL-17 in the BD patients with active uveitis were lower than the patients with inactive

**Table 2**
Primers sequence and PCR condition.

| Primers | Sequence | $T_m$ | Product Size | PCR condition | |
|---|---|---|---|---|---|
| GAPDH-F | ACAACTTTGGTATCGTGGAAGG | 58.59 | 101 | 1 time | 95 °C, 15 min |
| GAPDH-R | GCCATCACGCCACAGTTTC | 59.79 | | 40 times | 95 °C, 20 s |
| IL17A-F | AGATTACTACAACCGATCCACCT | 59.79 | 151 | | 58.5 °C, 30 s |
| IL17A-R | GGGGACAGAGTTCATGTGGTA | 59.09 | | | |

PCR: polymerase chain reaction; F: Forward; R: Reverse; min: minute; s: second.

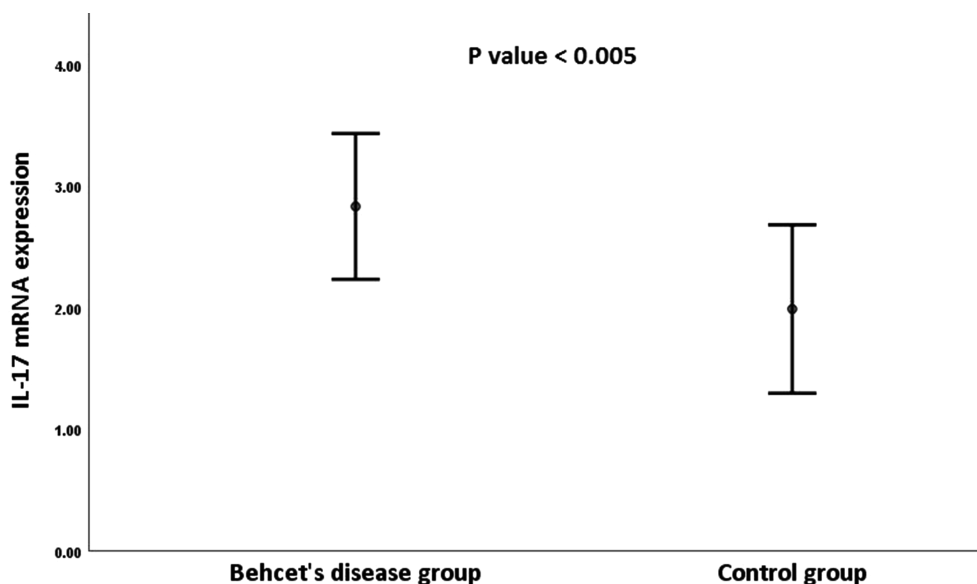



**Fig. 1.** IL-17 mRNA expression in Behçet's disease patients compared with healthy controls. The Mann–Whitney *U* test were used to make a comparison between the variables. Significant difference was observed in IL-17 mRNA expression between Behcet's disease patients and healthy controls ($P < 0.005$).

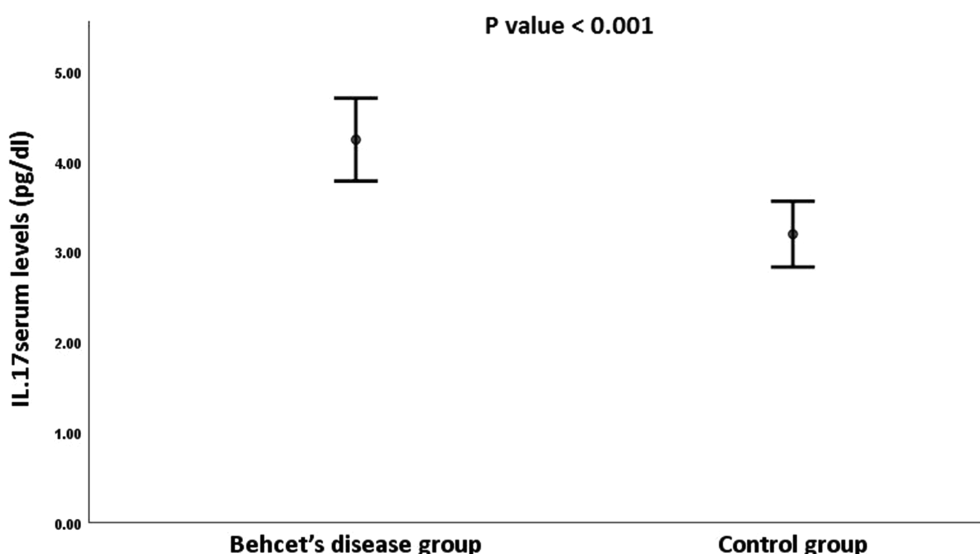



**Fig. 2.** Serum levels of IL-17 (detected by ELISA) in Behçet's disease patients compared with healthy controls. The independent *t*-test was used to make a comparison between the mean values. Significant difference was observed in IL-17 serum levels between Behcet's disease patients (mean $4.24 \pm 1.55$) and healthy controls ($3.19 \pm 1.53$; $P < 0.001$).

uveitis. There was no association between other clinical manifestations of BD and these parameters. In addition, we could not find a significant correlation between BDCAF and IBDDAM with IL-17 mRNA expression and serum levels (Table 4). However, TIAI had a significant and negative correlation with serum levels of IL-17 (Table 4).

## 4. Discussion

In the present study, we measured IL-17 mRNA expression and serum levels in patients with BD. We found a higher IL-17 mRNA expression and serum levels in our BD patients compared with the healthy controls. Higher IL-17 mRNA expression and serum levels were observed in the BD group aged < 25 at disease onset, positive pathergy test, and positive HLA-B5 and HLA-B51. Lower serum IL-17 levels were found in the BD patients with active uveitis compared to the BD patients with inactive uveitis. No association was observed between IL-17 mRNA expression and serum levels with mucocutaneous lesions, arthritis, and phlebitis. No significant correlation was observed between BD activity and IL-17 mRNA expression and serum levels, except TIAI which had a negative correlation with IL-17 serum levels.

**Table 3**
Association between demographic, clinical and laboratory features of Behçet's disease patients and IL-17 mRNA expression and serum levels.

| Demographic, clinical and laboratory features | Frequency | IL-17 mRNA expression rate (mean ± SD) | P-value | Serum IL-17 (mean ± SD) | P-value |
|---|---|---|---|---|---|
| Age at disease onset (years) | | | | | |
| < 25 | 25 (54.3) | 3.20 ± 2.13 | 0.213 | 4.76 ± 1.41 | 0.022 |
| ≥25 | 21 (45.7) | 2.48 ± 1.83 | | 3.63 ± 1.54 | |
| Gender | | | | | |
| Male | 29 (63) | 2.95 ± 1.95 | 0.393 | 4.32 ± 1.61 | 0.942 |
| Female | 17 (37) | 2.62 ± 2.16 | | 4.10 ± 1.45 | |
| Oral ulcer | | | | | |
| Yes (%) | 44 (95.7) | 2.86 ± 2.02 | – | 4.22 ± 1.55 | – |
| No (%) | 2 (4.3) | 2.06 ± 2.51 | | 4.61 ± 1.96 | |
| Genital ulcer | | | | | |
| Yes (%) | 23 (50) | 2.97 ± 2.25 | 0.818 | 4.16 ± 1.53 | 0.809 |
| No (%) | 23 (50) | 2.69 ± 1.79 | | 4.31 ± 1.59 | |
| Positive Pathergy test | | | | | |
| Yes (%) | 14 (30.4) | 4.20 ± 1.38 | 0.018 | 5.30 ± 0.99 | 0.002 |
| No (%) | 32 (69.6) | 2.54 ± 2.03 | | 3.55 ± 1.40 | |
| Uveitis | | | | | |
| Yes (%) | 35 (76.1) | 2.96 ± 2.04 | 0.422 | 4.37 ± 1.62 | 0.286 |
| No (%) | 11 (23.9) | 2.42 ± 2.04 | | 3.80 ± 1.29 | |
| Active Uveitis | | | | | |
| Yes (%) | 24 (68.6) | 2.70 ± 2.01 | 0.242 | 3.87 ± 1.51 | 0.009 |
| No (%) | 11 (31.4) | 3.58 ± 1.91 | | 5.31 ± 1.42 | |
| Pseudofolliculitis | | | | | |
| Yes (%) | 11 (23.9) | 3.53 ± 1.74 | 0.124 | 4.73 ± 1.06 | 0.320 |
| No (%) | 35 (76.1) | 2.61 ± 2.07 | | 4.08 ± 1.65 | |
| Arthritis | | | | | |
| Yes (%) | 9 (7.8) | 1.64 ± 1.47 | 0.067 | 3.95 ± 1.40 | 0.549 |
| No (%) | 37 (31.9) | 3.12 ± 2.04 | | 4.31 ± 1.58 | |
| Erythema Nodosum | | | | | |
| Yes (%) | 9 (19.6) | 2.52 ± 2.08 | 0.547 | 4.26 ± 1.31 | 0.978 |
| No (%) | 37 (80.4) | 2.90 ± 2.02 | | 4.23 ± 1.61 | |
| Phlebitis | | | | | |
| Yes (%) | 6 (13) | 3.68 ± 1.57 | 0.215 | 5.33 ± 0.95 | 0.064 |
| No (%) | 40 (87) | 2.70 ± 2.06 | | 4.07 ± 1.55 | |
| HLA-B5 | | | | | |
| Yes (%) | 27 (65.4) | 3.66 ± 2.07 | 0.041 | 4.69 ± 1.45 | 0.021 |
| No (%) | 19 (34.6) | 2.66 ± 2.59 | | 3.18 ± 1.46 | |
| HLA-B51 | | | | | |
| Yes (%) | 25 (53.3) | 4.21 ± 1.78 | 0.020 | 5.53 ± 1.08 | 0.006 |
| No (%) | 21 (46.7) | 2.06 ± 2.03 | | 2.96 ± 1.51 | |

SD, standard deviation; HLA, human leukocyte antigen.

IL-17, which is mainly produced by Th17 cells, is a strong pro-inflammatory cytokine that mobilizes and activates neutrophils and macrophages [9]. IL-17-family consists of 6 ligands [IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F] [33]. IL-17 induces the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α, chemokines like IL-8, CXCL1 and monocyte chemoattractant protein-1 (MCP-1), and prostaglandins from endothelial cells, epithelial cells and macrophages [33]. For this reason, it is believed that Th17 cells and IL-17 may be involved in the pathogenesis of inflammation in the acute phase of BD. Several studies emphasize the importance of Th17 cells and Th17-mediated cytokines in the inflammatory phase and tissue injury in patients with BD. Kim et al. showed higher Th17/Th1 ratio in BD patients in comparison with RA patients and healthy controls [34]. They found higher Th17/Th1 ratio in BD patients with uveitis and folliculitis compared to other BD patients. Hamzaoui et al. studied RORC (Th17-associated transcription factor) expression in the cerebrospinal fluid of 18 patients with Neuro-Behçet's Disease (NBD), 16 patients with non-inflammatory neurological disease and 10 patients with headaches attributed to BD [25]. They found higher expression of RORC in NBD patients compared with controls. Chi et al. in a study on 23 patients with BD showed higher expression of IL-17 by PBMCs in patients with uveitis compared with patients without uveitis and also healthy controls [22]. Another study on 8 BD patients with active uveitis, 8 BD patients with inactive uveitis and 8 healthy controls showed a higher production of IL-17 by $CD4^+$ T cells in BD patients with active uveitis [35]. Ekinci et al. reported a significantly higher serum IL-17A in patients with active BD (28.7 ± 12.8 pg/mL) compared with patients with inactive BD (11.9 ± 2.7 pg/mL) and healthy controls. Serum IL-17A levels were significantly higher in BD patients with active uveitis (29.8 ± 18.2 pg/mL) compared with those of BD patients with uveitis in remission (11.3 ± 2.9 pg/mL) [23]. In another study, Hamzaoui et al. assessed plasma IL-17 levels and the expression of RORC in patients with active BD, patients with inactive BD and healthy controls [24]. They found higher plasma IL-17 levels and Th17 in PBMCs of patients with active BD. Chi et al., in a study showed the importance of IL23/IL17 pathway in the pathogenesis of uveitis [22]. They found an increased production of IL-23 and IL-17 and promotion of IL-17 production by PBMCs in BD patients with uveitis. Contrary to other studies, in the study of Emiroglu et al. in 76 patients with active BD, there was no significant difference in IL-17A level compared to 70 patients with inactive BD [36]. No associations were observed between the serum IL-17A level and various clinical manifestations of BD. Ferrante et al. in a study compared the Th1 and Th17 axis cytokines in the mucosal biopsy and serum of patients with active BD, AS and Crohn's disease (CD) with healthy controls [37]. All patients had gastrointestinal symptoms. Th1 cytokines like IL-12, TNF-α and IFN-γ in the serum and ileum mucosal specimens of BD patients were higher than AS patients and healthy controls. In contrast IL-23 and IL-17 in BD patients were lower than patients with AS and CD. No differences were observed between BD patients and healthy controls. They concluded that Th17 axis has no role in the pathogenesis of the gastrointestinal attacks of BD.

Our results support the previous studies that suggest the role of IL-17 in the pathogenesis of BD. However, in contrast to previous studies, we found no correlation between IL-17 mRNA expression and serum levels with BD activity. Similarly, we found no association between IL-17 status and organ involvement except the higher serum IL-17 in patients with positive pathergy test and inactive uveitis. Although the underlying mechanism of pathergy phenomenon is not clear, mononuclear cells' infiltration around dermal vessels at the pathergy site and neutrophils in the infiltrate were reported [38]. Hyperactivity of neutrophils, which is one of the BD features plays an important role in the pathogenesis of pathergy phenomenon [38]. The pro-inflammatory effect of IL-17 may play a role in this phenomenon. Although cytokines

**Table 4**
Correlation between IL and 17 mRNA expression and serum levels with Behçet's disease activity.

| | BDCAF | | IBDDAM | | TIAI | |
|---|---|---|---|---|---|---|
| | Pearson correlation | P-value | Pearson correlation | P-value | Pearson correlation | P-value |
| IL-17 mRNA expression | −0.241 | 0.107 | 0.045 | 0.769 | −0.104 | 0.491 |
| Serum IL-17 levels (pg/ml) | −0.272 | 0.069 | −0.177 | 0.246 | −0.452 | 0.006 |

BD, Behçet's disease; BDCAF, Behçet's disease current activity form; IBDDAM, Iranian Behçet's disease dynamic activity measure; TIAI, Total inflammatory activity index.

play an important role in the pathogenesis of inflammatory disorders because of their vast pro- and anti-inflammatory effects, it is often difficult to use cytokines as prognostic or diagnostic tools. One of the most important reasons is the overlap between normal and abnormal cytokine levels [39]. A few studies investigated cytokines in healthy individuals and no clear cut-off value exists for cytokines. Many factors contribute to cytokine release and action [39]. Lack of positive correlation between IL-17 serum levels with BD activity and negative correlation between these biomarker and uveitis attacks suggest that a Th1, but not a Th17, response may be present in the inflammatory BD attacks.

## 5. Conclusions

Although the Th17 response may contribute to the pathogenesis of BD, IL-17 mRNA expression rate and serum levels cannot be used as a biomarker in assessing BD activity and predicting various organs' involvement.

## Author contributions

a. Golamreza Jadideslam: Design of the study, drafting of the manuscript, analyzing the interleukin-17 mRNA expression and serum levels

b. Houman Kahroba: Drafting of the manuscript, Critical revision of the manuscript for important intellectual content

c. Khalil Ansarin: Drafting of the manuscript, Critical revision of the manuscript for important intellectual content

d. Ebrahim Sakhinia: Supervision on analyzing the interleukin-17 mRNA expression and serum levels, Critical revision of the manuscript for important intellectual content.

e. Alireza Abhar: Analyzing data

F. 6. Shahriar Alipour: Data collection, Critical revision of the manuscript for important intellectual content

F. Jafar Farhadi: Data collection, Critical revision of the manuscript for important intellectual content

G. Sam Seydi Shirvani: Data collection, Critical revision of the manuscript for important intellectual content

H. Masoud Nouri-Vaskeh: Data collection, Critical revision of the manuscript for important intellectual content

I. Saeed Mousavi: Statistical analysis, Drafting of the manuscript

J. Alireza khabbazi: Design and supervision of the study, Critical revision of the manuscript for important intellectual content

All the authors have read and approved the final version of the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was sponsored by a grant from the faculty of Advanced Medical Sciences and Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences.

## References

[1] B. Tong, X. Liu, J. Xiao, G. Su, Immunopathogenesis of Behçet's disease, Front. Immunol. 10 (2019) 665, https://doi.org/10.3389/fimmu.2019.00665.

[2] P. Leccese, E. Alpsoy, Behçet's disease: an overview of etiopathogenesis, Front. Immunol. 10 (2019) 1067, https://doi.org/10.3389/fimmu.2019.01067.

[3] S. Alipour, M. Nouri, E. Sakhinia, N. Samadi, N. Roshanravan, A. Ghavami, A. Khabbazi, Epigenetic alterations in chronic disease focusing on Behçet's disease, Rev., Biomed. Pharmacother. = Biomed. Pharmacother. 91 (2017) 526–533, https://doi.org/10.1016/j.biopha.2017.04.106.

[4] S. Kolahi, M.J. Farajzadeh, S. Alipour, A. Abhari, J. Farhadi, N. Bahavarnia, A. Malek Mahdavi, A. Khabbazi, E. Sakhinia, Determination of mir-155 and mir-146a expression rates and its association with expression level of TNF-alpha and CTLA4 genes in patients with Behçet's disease, Immunol. Lett. 204 (2018) 55–59, https://doi.org/10.1016/j.imlet.2018.10.012.

[5] A. Hosseini, D. Shanehbandi, M.A. Estiar, S. Gholizadeh, A. Khabbazi, H. Khodadadi, E. Sakhinia, Z. Babaloo, A single nucleotide polymorphism in the FOXP3 gene associated with Behçet's disease in an Iranian population, Clin. Lab. 61 (12) (2015) 1897–1903.

[6] K. Hamzaoui, Th17 cells in Behçet's disease: a new immunoregulatory axis, Clin. Exp. Rheumatol. 29 (4 Suppl 67) (2011) S71–S76.

[7] G. Di Scala, A. Bettiol, R.D. Cojan, M. Finocchi, E. Silvestri, G. Emmi, Efficacy of the anti-IL 17 secukinumab in refractory Behçet's syndrome: a preliminary study, J. Autoimmun. 97 (2019) 108–113, https://doi.org/10.1016/j.jaut.2018.09.002.

[8] G. Geri, B. Terrier, M. Rosenzwajg, B. Wechsler, M. Touzot, D. Seilhean, T.A. Tran, B. Bodaghi, L. Musset, V. Soumelis, D. Klatzmann, P. Cacoub, D. Saadoun, Critical role of IL-21 in modulating TH17 and regulatory T cells in Behçet' disease, J. Allergy Clin. Immunol. 128 (3) (2011) 655–664, https://doi.org/10.1016/j.jaci.2011.05.029.

[9] L.A. Tesmer, S.K. Lundy, S. Sarkar, D.A. Fox, Th17 cells in human disease, Immunol. Rev. 223 (2008) 87–113, https://doi.org/10.1111/j.1600-065X.2008.00628.x.

[10] W. Kaabachi, E. Bouali, A. Berraies, I.B. Dhifallh, B. Hamdi, K. Hamzaoui, A. Hamzaoui, Interleukin-26 is overexpressed in Behçet's disease and enhances Th17 related -cytokines, Immunol. Lett. 190 (2017) 177–184, https://doi.org/10.1016/j.imlet.2017.08.008.

[11] W. Kaabachi, M. Khaouthar, B. Hamdi, I. Khalfallah, J. Ammar, K. Hamzaoui, A. Hamzaoui, Th 9 cells in Behçet' disease: Possible involvement of IL-9 in pulmonary manifestations, Immunol. Lett. 211 (2019) 3–12, https://doi.org/10.1016/j.imlet.2019.05.004.

[12] M. Ziolkowska, A. Koc, G. Luszczykiewicz, K. Ksiezopolska-Pietrzak, E. Klimczak, H. Chwalinska-Sadowska, W. Maslinski, High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism, J. Immunol. (Baltimore, Md. 1950) 164 (5) (2000) 2832–2838, https://doi.org/10.4049/jimmunol.164.5.2832.

[13] S. Fujino, A. Andoh, S. Bamba, A. Ogawa, K. Hata, Y. Araki, T. Bamba, Y. Fujiyama, Increased expression of interleukin 17 in inflammatory bowel disease, Gut 52 (1) (2003) 65–70, https://doi.org/10.1136/gut.52.1.65.

[14] K. Kurasawa, K. Hirose, H. Sano, H. Endo, H. Shinkai, Y. Nawata, K. Takabayashi, I. Iwamoto, Increased interleukin-17 production in patients with systemic sclerosis, Arthritis Rheum. 43 (11) (2000) 2455–2463, https://doi.org/10.1002/1529-0131(200011)43:11<2455::Aid-anr12>3.0.Co;2-k.

[15] S. Mojaverrostami, M.N. Bojnordi, M. Ghasemi-Kasman, M.A. Ebrahimzadeh, H.G. Hamidabadi, A review of herbal therapy in multiple sclerosis, Adv. Pharm. Bull. 8 (4) (2018) 575–590, https://doi.org/10.15171/apb.2018.066.

[16] B. Astry, S.H. Venkatesha, K.D. Moudgil, Involvement of the IL-23/IL-17 axis and the Th17/Treg balance in the pathogenesis and control of autoimmune arthritis, Cytokine 74 (1) (2015) 54–61, https://doi.org/10.1016/j.cyto.2014.11.020.

[17] D. Baeten, J. Sieper, J. Braun, X. Baraliakos, M. Dougados, P. Emery, A. Deodhar, B. Porter, R. Martin, M. Andersson, S. Mpofu, H.B. Richards, Secukinumab, an interleukin-17A inhibitor, ankylosing spondylitis, N. Engl. J. Med. 373 (26) (2015) 2534–2548, https://doi.org/10.1056/NEJMoa1505066.

[18] I.B. McInnes, P.J. Mease, B. Kirkham, A. Kavanaugh, C.T. Ritchlin, P. Rahman, D. van der Heijde, R. Landewe, P.G. Conaghan, A.B. Gottlieb, H. Richards, L. Pricop, G. Ligozio, M. Patekar, S. Mpofu, Secukinumab, a human anti-interleukin-17A monoclonal antibody, in patients with psoriatic arthritis (FUTURE 2): a randomised, double-blind, placebo-controlled, phase 3 trial, Lancet (London, England) 386 (9999) (2015) 1137–1146, https://doi.org/10.1016/s0140-6736(15)61134-5.

[19] A. Amadi-Obi, C.R. Yu, X. Liu, R.M. Mahdi, G.L. Clarke, R.B. Nussenblatt, I. Gery, Y.S. Lee, C.E. Egwuagu, TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1, Nat. Med. 13 (6) (2007) 711–718, https://doi.org/10.1038/nm1585.

[20] W. Chi, P. Yang, B. Li, C. Wu, H. Jin, X. Zhu, L. Chen, H. Zhou, X. Huang, A. Kijlstra, IL-23 promotes CD4+ T cells to produce IL-17 in Vogt-Koyanagi-Harada disease, J. Allergy Clin. Immunol. 119 (5) (2007) 1218–1224, https://doi.org/10.1016/j.jaci.2007.01.010.

[21] H. Shen, L.P. Xia, J. Lu, Elevated levels of interleukin-27 and effect on production of interferon-gamma and interleukin-17 in patients with Behçet's disease, Scand. J. Rheumatol. 42 (1) (2013) 48–51, https://doi.org/10.3109/03009742.2012.704391.

[22] W. Chi, X. Zhu, P. Yang, X. Liu, X. Lin, H. Zhou, X. Huang, A. Kijlstra, Upregulated IL-23 and IL-17 in Behçet' patients with active uveitis, Invest. Ophthalmol. Vis. Sci. 49 (7) (2008) 3058–3064, https://doi.org/10.1167/iovs.07-1390.

[23] N.S. Ekinci, E. Alpsoy, A.A. Karakas, S.B. Yilmaz, O. Yegin, IL-17A has an important role in the acute attacks of Behçet's disease, J. Invest. Dermatol. 130 (8) (2010) 2136–2138, https://doi.org/10.1038/jid.2010.114.

[24] K. Hamzaoui, E. Bouali, I. Ghorbel, M. Khanfir, H. Houman, A. Hamzaoui, Expression of Th-17 and RORgammat mRNA in Behçet's Disease, Med. Sci. Monit.: Int. Med. J. Exp. Clin. Res. 17 (4) (2011) Cr227-34, https://doi.org/10.12659/msm.881720.

[25] K. Hamzaoui, A. Borhani Haghighi, I.B. Ghorbel, H. Houman, RORC and Foxp3 axis in cerebrospinal fluid of patients with neuro-Behçet's disease, J. Neuroimmunol. 233 (1–2) (2011) 249–253, https://doi.org/10.1016/j.jneuroim.2011.01.012.

[26] K. Hamzaoui, A. Hamzaoui, F. Guemira, M. Bessioud, M. Hamza, K. Ayed, Cytokine profile in Behçet's disease patients. Relationship with disease activity, Scand. J. Rheumatol. 31 (4) (2002) 205–210.

[27] J. Shimizu, K. Takai, E. Takada, N. Fujiwara, N. Arimitsu, Y. Ueda, S. Wakisaka, T. Suzuki, N. Suzuki, Possible association of proinflammatory cytokines including IL1beta and TNFalpha with enhanced Th17 cell differentiation in patients with Behçet's disease, Clin. Rheumatol. 35 (7) (2016) 1857–1863, https://doi.org/10.1007/s10067-015-2966-2.

[28] I.T.f.t.R.o.t.I.C.f.B.s.D. (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.: JEADV 28(3) (2014) 338–47. doi:10.1111/jdv.12107.

[29] F. Davatchi, Iran Behçet's disease dynamic activity measure. Abstracts of the XIIth European congress of rheumatology, Hungarian Rheumatol. 32 (1991) 134.

[30] G. Lawton, B.B. Bhakta, M.A. Chamberlain, A. Tennant, The Behçet's disease activity index, Rheumatology (Oxford, England) 43 (1) (2004) 73–78, https://doi.org/10.1093/rheumatology/keg453.

[31] F. Shahram, A. Khabbazi, A. Nadji, N. Ziaie, A.T. Banihashemi, F. Davatchi, Comparison of existing disease activity indices in the follow-up of patients with Behçet's disease, Mod. Rheumatol. 19 (5) (2009) 536–541, https://doi.org/10.1007/s10165-009-0191-z.

[32] S.Y. Na, M.J. Park, S. Park, E.S. Lee, Up-regulation of Th17 and related cytokines in Behçet's disease corresponding to disease activity, Clin. Exp. Rheumatol. 31 (3 Suppl 77) (2013) 32–40.

[33] R.M. Onishi, S.L. Gaffen, Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease, Immunology 129 (3) (2010) 311–321, https://doi.org/10.1111/j.1365-2567.2009.03240.x.

[34] J. Kim, J. Park, E. Lee, Y. Lee, Y. Song, E. Lee, Imbalance of Th17 to Th1 cells in Behçet's disease, Clin. Exp. Rheumatol. 28 (4 Suppl 60) (2010) S16–S19.

[35] W. Chi, S. Zhou, P. Yang, L. Chen, CD4(+) T cells from Behçet' patients produce high levels of IL-17, Eye Sci. 26 (2) (2011) 65–69, https://doi.org/10.3969/j.issn.1000-4432.2011.02.013.

[36] N. Emiroglu, F.P. Cengiz, G.B. Erdem, Serum IL-17A in Behçet's disease, Postepy dermatologii i alergologii 32 (5) (2015) 358–361, https://doi.org/10.5114/pdia.2015.54745.

[37] A. Ferrante, F. Ciccia, A. Principato, A.R. Giardina, R. Impastato, S. Peralta, G. Triolo, A Th1 but not a Th17 response is present in the gastrointestinal involvement of Behçet's disease, Clin. Exp. Rheumatol. 28 (4 Suppl 60) (2010) S27–S30.

[38] O. Gunduz, Histopathological evaluation of Behçet's disease and identification of new skin lesions, Pathol. Res. Int. 2012 (2012) 209316, , https://doi.org/10.1155/2012/209316.

[39] R.N. Monastero, S. Pentyala, Cytokines as biomarkers and their respective clinical cutoff levels, Int. J. Inflam. 2017 (2017) 4309485, https://doi.org/10.1155/2017/4309485.