RABMS
International Journal of
Research in Applied and
Basic Medical Sciences
ISSN: 2476-3624

Determination of the relationship between the severity of Behcet's disease and the expression and methylation of IL-10, IL-6 and IL-8 genes

Somayeh Abolhasani [1], Alireza Khabbazi [2], Shiva Gholizadeh-Ghaleh Aziz [1*], Shahriar Alipour [1*]

[1] Department of clinical biochemistry, faculty of medicine, medical university of Urmia, Iran

2 Connective Tissue Disease, Tabriz University of Medical Science, Tabriz, Iran

***Corresponding authors:** Shahriar Alipour, Shiva Gholizadeh-Ghaleh Aziz, **Address:** Department of Clinical Biochemistry; Faculty of Medicine; Urmia University of Medical Sciences; Urmia; Iran, **Email**: alipoursh@umsu.ac.ir, gholizadeh.sh@umsu.ac.ir, **Zip Code**: 5756115111, **Tel/Fax**: +984432780803

Abstract

***Background & Aims*:** The immune system interference in Behcet's disease (BD) is typically cleared by Th1 as well as Th17 pathway - mediated inflammatory process.Among these, Interleukin-10 and 6, 8 as cytokines involved in inflammation have played a major role in autoimmune diseases. The aim of this study was to investigate the relationship between the severity of Behcet's disease with gene expression and methylation of IL-10, IL-6 and IL -8 genes.

***Materials and Methods*:** Since this project had been investigated in a large study, we examined the association of IL10, IL6 and IL8 genes with the severity of BD. Accordingly, all analyses in this paper are limited to people with Behcet's. In this study, 26 patients are with severe BD and 21 with no severe BD. So, we examined Relationship between severity of BD disease and expression and methylation in these genes through appropriate statistical methods.

***Results*:** The mean expression rate of IL6 and IL8 genes in positive subjects with severe BD is higher than that of the ones with no severe BD; however, this is opposite in IL10. In addition, the methylation level of IL6 and IL10 genes in no severe BD subjects is greater than that of those with severe BD, however it is opposite in IL8.The difference between the expressions of IL10 in two groups and the difference between the methylation levels of IL8 and IL10 genes were reported to be significant ($P < 0.05$).

***Conclusion*:** In general, our results did not suggest any correlation between gene expressions of IL-10, Il-6, and IL-8. However, expression and methylation of these genes in the studied groups were difference in terms of disease severity.

***Key words*:** Behcet's disease, severity, IL-10, IL-6, IL-8, Methylation levels

Received 11 Feb 2018, accepted for publication 22 Apr 2018

Introduction

Behcet's disease (BD) or Behcet's syndrome (BS) is an autoinflammatory chronic vasculitis disorder which almost 80% of patients were from the Middle East (Silk Road regions) (1); despite much researchis done for effectively managed it, but its etiology still is undiscovered completely. BD has the main recurrent sings in several organs such as ulcer in genital and ophthalmic and ocular systems and there are strong elements to discriminate BD which include oral ulcers, eye disease, genital ulceration, major vascular involvement, parenchymal neurological disease(2). The immune system interference in BD is typically cleared by Th1 as well as Th17 pathway - mediated

inflammatory process (3) by elevation levels of IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 and TNF-α (1, 2).

Interleukin-6 (IL-6) as a pre-inflammatory and multifunctional cytokine, produced by lymphocyte and macrophages, has a wide-ranging of biologic activities to simultaneous immune system to production of lymphocyte and acute phase protein and induction of T cell growth to providing CD8 for cytotoxic activity(4). The gene of this cytokine is located on chromosome 7p15.3. Elevation the other cytokines level to elevating the inflammation(1). Insufficient of IL-6 gene expression contributes to the induction and incidence of several diseases, such as cancers and autoimmune disease(5). IL-6 be active mainly via the JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogenactivated protein kinase) signaling pathways in BD(6).

Interleukin-8 (IL-8), gene location is on chromosome 4q13.3, has the critical role in two statuses of the disease; the beginning and sustained inflammatory motivation. Monocytes secrete IL-8subsequent lipopolysaccharide (LPS) stimulation(7). The induction rate growth of T-cells by acute phase protein (heat shock proteins) influence on the proinflammatory cytokine mRNAs expression, IL-8, and cause the damageat retinal and mucosal tissues(7, 8). One of the main reasons for inflammatory reactions in BD depends on the increasing of the proinflammatory cytokine gene expression (9).

Interleukin-10 (IL-10), unlike the both IL-6 and IL-8, is an anti-inflammatory cytokine that inhibits T cell function, differentiation and regulation by suppressing the production of TNFα, IL-1, IL-6, IL-8, and IL-12 (10)and avoiding the modification of T cells into T helper 17 cells (Th17) and T cells (Tregs) (10, 11). The homosapien IL-10 gene is located on chromosome 1q21–32. Several types of cell secret IL-10 such as T-helper type 2 (mainly), 1 and 17 (Th) cells, regulatory T cells (Tr1), cytotoxic T cells, dendritic cells, macrophages, monocytes, mast cells and B lymphocytes, NK cells, eosinophil (12). IL-10 via the Toll-like receptor, p38 and ERK, STAT1 and STAT3 signaling pathways start to anti-inflammatory activations(13). Therefore, according to the notes, the aim of this study was to investigate the relationship between the severity of Behcet's disease with gene expression and methylation of IL-10, IL-6 and IL -8 genes.

Materials and Methods

This case-control study was performed on 47 patients with Behcet and 61 healthy individualsduring 2015-2017. All patients were chosen from Azeri population of the country (people of Azerbaijan Province) and after a clinical examination by rheumatologists, they were introduced to the Molecular Laboratory of the Faculty of Medicine of Tabriz University of Medical Sciences in Urmia. Prior to entering the study, the written consent was obtained from all individuals. All referring patients were selected by IBDDAM[1] and ICBD[2]criteria. The studied volume was also calculated using the results of pilot studies by power & sample software. In this study, molecular investigations were performed on a number of genes involved in the disease;in this study, we examined the association of some of these genes with the severity of the disease. The patients' mean age was 38.02 ± 10.25 and the healthy subjects' mean age was 37.4 ± 8.5 and no significant differences were observed betweenthe two groups. In the present study, in terms of severity, 26 patients were with severe BD and 21 were negative for severe BD.

Blood samples were given from all subjects; then, single cells (PBMCs) were extracted from all of them, followed by DNA and RNR extraction for molecular analysis. Ultimately, the expression rate of the target

[1]Iranian Behcet's Disease Dynamic Activity Measure

[2]The International Criteria for Behcet's Disease

genes (IL6, IL8 and IL10) and the methylation level of these genes were assessed using Real-time PCR. Of course, to analyze the methylation level, the methylated and non-methylated positions were first determined by MeDIP method and then quantitatively measured. Since this project had been investigated in a large study, we examined the associationof IL10, IL6 and IL8 genes with the severity of Behcet disease. Accordingly, all analyses in this paper are limited to people with Behcet.

Results

Forty-seven patients with Behcet participated in this study, of whom 18 (38.3%) were female and 29 (61.7%) were male. The mean age of the subjects was 38.91 ± 10.24 years.

In this study, 26 patients arewith severe BD and 21 with no severe BD. The descriptive statistics of the expression and methylation rates of IL6, IL8 and IL10 genes are shown in Table 1. It is observed that the mean expression rate of IL6 and IL8 genes in positive subjects with severe BD is higher than that of the ones with no severe BD;however, this is opposite in IL10. In addition, the methylation level of IL6 and IL10 genes in no severe BD subjects is greater than that of those withsevere BD, however it is opposite in IL8. The difference between theexpressions of IL10 in two groups and the difference between the methylation levels of IL8 and IL10 genes were reported to be significant ($P < 0.05$). Comparison between the gene expression rate and the methylation level is shown in Figs. 1 and 2.

The analysis of correlation between gene expression andthe severity of disease indicated that correlation between gene expressions in all three genes was positive and insignificant, and the correlation between the methylation levels in all three genes was negative and insignificant.Also, the correlation between the amount of methylation in all three genes was negative and non-significant. The distribution chart for the correlation between the expression of genes and their methylation is shown in Figures 3 to 4.

Table 1. Comparison of Expression and Methylation in Severe BD and No Severe BD Groups

	Expression		Methylation	
	Severe BD	No Severe BD	Severe BD	No Severe BD
Interleukin 6	1.57±1.59	1.91±1.11	0.69±0.08	0.71±0.08
Interleukin 8	1.86±0.43	2.13±0.51	0.56±0.11	0.49±0.10
Interleukin 10	1.51±0.28	1.22±0.07	0.72±0.09	0.82±0.08

Table 2. Correlations between Expression of Interleukin 6, 8 and 10 in Patient with Severe BD

		Interleukin 6		Interleukin 8		Interleukin 10	
		r	P-value	r	P-value	r	P-value
Expression	Interleukin 6	-----		-0.090	0.662	0.308	0.126
	Interleukin 8	-----		-----		0.034	0.867
Methylation	Interleukin 6	-----		-0.164	0.423	-0.269	0.184
	Interleukin 8	-----		-----		-0.049	0.812

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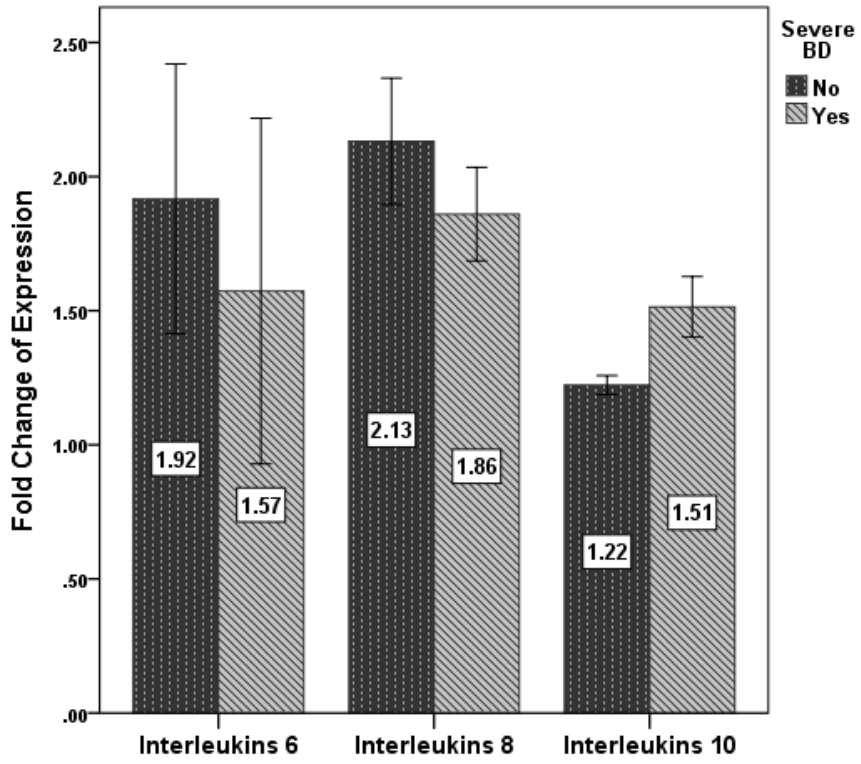

Figure 1. Comparison of Expression in Severe BD and No Severe BD Groups

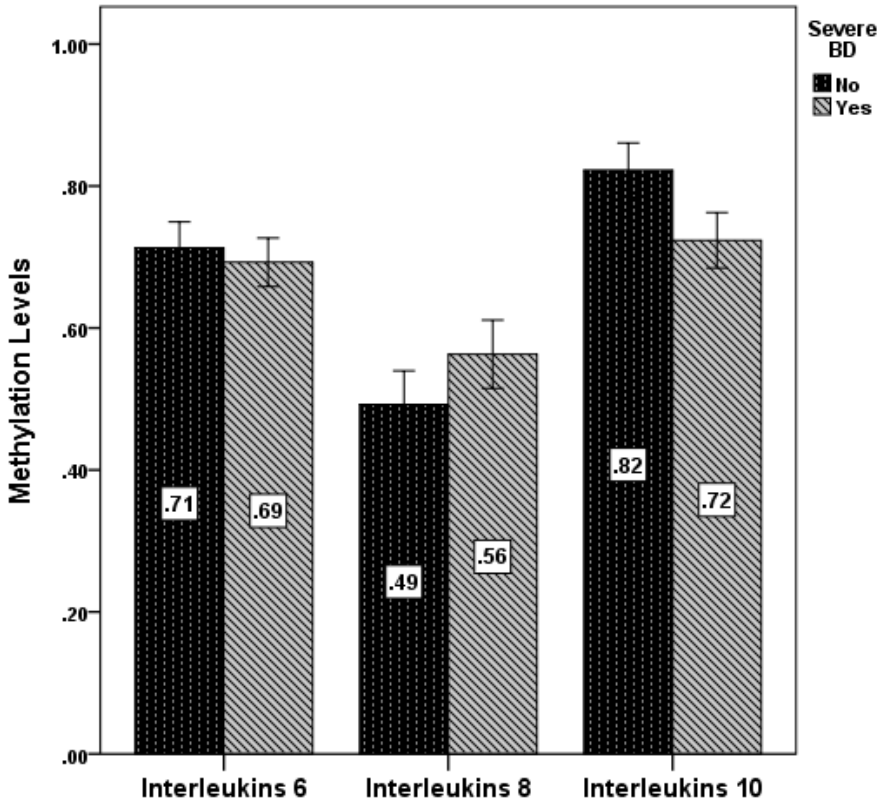

Figure 2. Comparison of Methylation in Severe BD and No Severe BD Groups

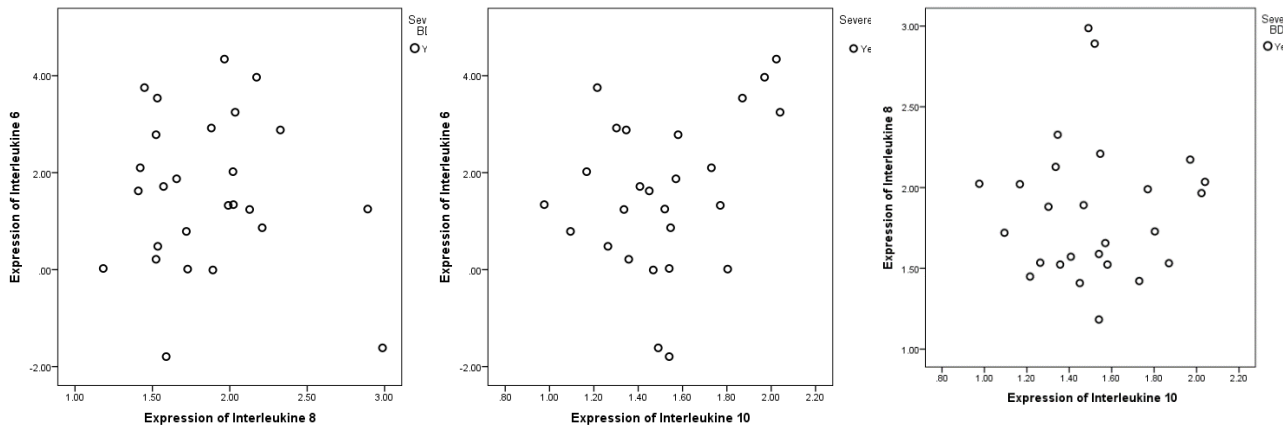

Figure 3. Correlation between Expression of Interleukin 6, Interleukin 10 and Interleukin 8

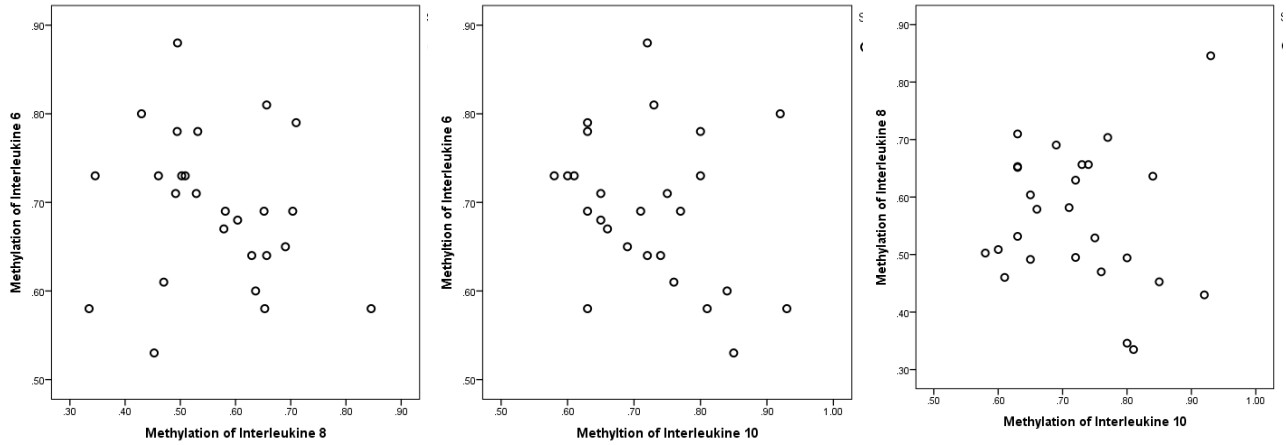

Figure 4. Correlation between Methylation of Interleukin 6, Interleukin 10 and Interleukin 8

Discussion

Nowadays, epigenetics is considered as an important mechanism in finding the causes of most diseases. Unusual methylation of CpG islands in relation with promoter can lead to the loss of gene expression which is an alternative mechanism of gene deactivation in mutations with function reduction. Over the past decades, epigenetic studies have been mostly focused on embryogenesis, aging, and cancer (14-16). However recently, epigenetic studies have been focused on many other fields such as inflammation, immune disease, obesity, insulin resistance, diabetes mellitus type 2, cardiac diseases, and neural analysis disease (17-19). These diseases are caused by epigenetic changes by inner and outer factors and their ability of changing gene expression. Cytokines and signal transduction pathways are innately related to other regions in cancer growth, distinction, maturity, and activation of the cells involved in inflammation and immunity. Regulation of inflammatory cytokine networks by epigenetic mechanism has been proved in many studies (20, 21).

As mentioned in the previous sections, in this study DNA methylation was used for reviewing the epigenetic mechanisms. For this purpose, in this research we studied the methylation changes of promoter role of the genes IL-10, Il-6, and IL-8. According to the previous studies, IL-10 acts as an inhibitor protein and IL-6 and Il-8 act as stimulant proteins (22-25). Also, it has been found that in most of the autoimmune diseases, expression of the mentioned genes is affected by various factors such as environmental, genetic, and infectious factors and changes significantly (25). Meanwhile, it has

been found that in most of these diseases, expression of these genes is correlated with some of the clinical symptoms of disease (26). Also, epigenetic studies on different populations have shown that there is a relationship between clinicopathologic factors and genome methylation pattern (27). Although in most of the autoimmune diseases such as Behcet's disease it has been proved that the expression of these genes is related to the disease procedure, no study has been done on the epigenetic role of regulation of expression of these genes in Behcet's disease and their relationship with clinical symptoms. Therefore, in this research, the relationship of the genes' expression and their methylation with their clinical symptoms, particularly with the severity of Behcet's disease were investigated.

The results of our previous studies showed that compared with the healthy group, expression of IL-10, Il-8, and IL-6 genes in the patients group were respectively decreased, decreased, and increased (22, 23). Whereas, the expression of these genes was decreased among the people with severe BD compared with the people without severe BD. However, this relationship was negative about Il-8 gene, i.e. in it was increased in people with severe BD compared with the people without severe BD. Also, in our study, promoter methylation of IL-10 and IL-8 genes was hypermethylated in the patients group and hypomethyalted in the healthy group. Whereas, methylation of IL-6 was quite negative and it was decreased in the patient group compared with the healthy group (22, 23), while the methylation of interleukin 6 and 10 genes in the people with no severe BD was higher than the people with severe BD, but in interleukin 8, this relationship ratio is negative. The difference between gene expression of interleukin 10 in the two groups and the difference between methylation of interleukin 8 and 10 genes was reported significant ($P<0.05$). Meanwhile, the results of correlation between gene expression and methylation of the mentioned genes revealed that the correlation between the gene expressions is positive and insignificant for all the three genes and the correlation between methylations is negative and insignificant for all the three genes.

Since the changes of small vessel walls are considered as the early pathologic symptom of this disease, interleukin 8 is the main known chemokine in active leukocytes (28) which is considered as a powerful mediator between active immune system and endothelial changes in active Behcet's disease. Increased level of interleukin 8 in patients with active Behcet's disease has been observed on the serum produced by peripheral monocytes, neutrophils, and T cells existing in damaged skin (29). In the study done by Zouboulis et al. in 2000 on Behcet's patients, it was found that compared with the patients whose disease has not yet become activated in patients with active Behcet's disease, increased level of serum interleukin 8 is observed, especially in patients with oral wounds and nervous problems and the results of this study are consistent with the results of our study (30).

Interleukin 6 is multifunctional cytokine which is produced and released by inflammatory cell and it can stimulate intracellular transcription factors and cause gene expression, cell division, apoptotic processes, and inflammatory responses (31, 32). Sustainable differences in IL-6 production have been proved among the various people of different populations with polymorphism at 5' and genetic relationship of IL-6 polymorphism with infection, inflammation, and different immune disease (33). The results of the study done by Adam et al showed that serum level of IL-6 and CRP are increased in patients with activated Behcet's diseases and it is inconsistent with the results of our study (34). Also, the results of the study done by Akman-Demir et al. showed that in patients with chronic pNB, Il-6 level in CSF increased, although this increase was not significant. Also, BD patients had significant increase of IL-6 levels in CSF compared with the RRMS patients, SSPE patients, and patients with central nervous system disorder (35).

It has been clearly revealed that interleukin 10 is an important cytokine for inhibiting inflammation and

preventing its progress through Th1 immune responses (36). More than 75% of intrapersonal changes of interleukin production are related to its genetic variety (37). The amounts of interleukin 10 adjust the immune responses with the balance of inflammatory and humoral responses (38). Chronic inflammation and its related responses are considered as main mechanisms of autoimmune diseases. However, the main processes in which inflammation cause autoimmunity in these diseases are still uncertain.

Conclusion

In general, our results did not suggest any correlation between gene expressions of IL-10, Il-6, and IL-8. However, expression and methylation of these genes in the studied groups were difference in terms of disease severity. It means that among the patients with positive disease severity, gene expression and methylation were significantly different from the patients without severe disease (no sever BD). In this way, through more studies, appropriate diagnosis and treatment methods can be designed for patient with high severity of disease.

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