Received: 3 October 2017 | Accepted: 23 February 2018
DOI: 10.1002/jcb.26809

RESEARCH ARTICLE

WILEY Journal of Cellular Biochemistry

Hypermethylation of IL-10 gene is responsible for its low mRNA expression in Behçet's disease

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Abstract

Interleukin-10 (IL-10), produced generally by monocyte, T helper type 2 (Th2), and regulatory T cells (Treg), plays a central role in controlling inflammatory responses and regulating the immune response of the IL-10 mRNA expression. It is significantly down-regulated in many autoimmune diseases such as Behçet's disease; this is mostly associated with more aggressive complications. Nevertheless, the essential molecular process for its low expression has not been completely realized. The aim of this project was attempted to estimate the gene expression, promoter methylation, and protein levels to IL-10's down-regulated expression. In this study, blood samples from 51 (4 missed) patients and 63 (2 missed) healthy controls were taken, with the mononuclear cells isolated by the Ficoll Protocol. DNA and RNA were then subsequently extracted. Promoter methylation levels were evaluated by MeDIP-qPCR. Following this, the extracted RNA was converted to cDNA using the RT-PCR method, with the expression of IL-10 later evaluated by Real-time PCR. And then, serum levels of IL-10 were measured using ELISA method. As we expected, the expression level of the IL-10 gene was seen to significantly decline in the patient group in comparison to the control. Also, the rate of promoter methylation was significantly higher in the IL-10 mRNA low expression group (patient group) compared to its high expression group (healthy group) ($P < 0.001$). We revealed that hypermethylation of promoter region was the principal defect for the IL-10 mRNA low expression in patients with Behçet's disease.

KEYWORDS

Behçet's disease, DNA methylation, IL-10, MeDIP-qPCR

1 | INTRODUCTION

Behcet's is an autoinflammatory disease that is defined by Hulusi Behcet in 1937 as an inflammatory process of unrevealed etiology, categorized by recurrent aphthous stomatitis, uveitis, genital ulcers, and skin lesions.[1] Although Behçet's disease (BD) is an extensive and worldwide disease in different parts of the world, it has remarkable local differences, with the maximum of incidences in the Mediterranean, the Middle East, and the Far East which was locally called the Silk Road. The prevalence of Behçet's disease has been reported to be the most in Turkey, including 421 people per 10^5.[2] This disease is a genetically complex disorder with unknown etiology. Among all genetic factors, HLA-B51 has been confirmed as the strongest risk factor for BD, which was verified in various populations. More recently, studies have disclosed the association of many non-HLA genes with the BD, such as chemokine receptor 1 (CCR1),[3] Vitamin D Receptor (VDR),[4] IL-23,[5] IL-23R, and IL-12RB2,[6,7] fork head box P3 box P3 (Foxp3),[8] interleukin (IL)-2, IL-4, transforming growth factor (TGF)-beta,[9] IL-27,[10] and the small ubiquitin-like modifier 4 (SUMO4)[11] for BD.

However, most of the studies focused on transcriptional events and additional mechanisms that controlled genes expression. Epigenetic mechanisms altered gene expression without changing the DNA sequence.[12] Recent researches of cytokine gene expression have focused on epigenetic alterations, because the DNA methylation status at CpG sites is often related with cytokine expression changes.[13–15] Decreasing of methylation is related to chromatin's open position, but increasing methylation corresponds to chromatin's closed status.[16,17] Furthermore, the extent to which CpG islands are methylated associates with the level of cytokine expression. Whether the decrease of DNA methylation in IL-10 gene in the T cells of patients with BD can modify the performance of transcription factor.

Proinflammatory and anti-inflammatory agents are involved in the beginning and the development of BD. Pioneering studies demonstrated that various cytokine networks are closely implicated in the onset, evolution and organ damages of this disease; thus, a thorough survey for controlling mechanism of the inflammatory cytokines' production during the progression of BD is crucial.[18,19] Interleukin-10 (IL-10), produced mostly by monocytes, T helper type 2 (Th2), and regulatory T cells (Treg), plays an essential role in repressing inflammation and controlling the immune response.[20] IL-10 is a type II cytokine and the element of a group of cytokines that contain IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29.[21] IL-10 is an anti-inflammatory cytokine that has been associated in several immune-mediated inflammatory diseases.[22] The decreased level of IL-10 is associated with disease activity according to several previous studies.[18,20] Moreover, it seems that IL-10 plays a crucial role in BD by affecting other cytokines' signaling pathways. This immunoregulatory cytokine stimulates the development of B cells, but prevents T cell proliferation. IL-10 can prevent inflammation by declining the production of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and other agents; it also up-regulates the production of suppressors of cytokines, such as soluble TNF receptor.

Since epigenetic alterations are reversible, it provides new molecular targets for autoimmune disease therapy. Recently, it has been proved that 5-azacytidine (5-azaC) as an inhibitor of DNA methyltransferases induces demethylation of the upstream and proximal CpG sites of IL-10 gene and increases its expression in peripheral blood mononuclear cells (PBMCs) in rheumatoid arthritis patients.[23] Furthermore, another in vitro study indicated that the treatment of healthy $CD4^+$ T cells with the 5-azaC can induce IL-10 expression.[24,25] These results can be due to the fact that 5-azaC treatment increases the ability of transcription factors such as CREB, C/EBPβ GRE, CREB, and Sp1 for binding upstream region of the genes promoter and causes the demethylation of CpGs regions and increases mRNA expression.[18]

Recent evidence has shown that low IL-10 expression is involved in the pathogenesis of autoimmune diseases such as BD.[18] Assuming that there is a link between IL-10 reduction and inflammatory conditions in BD, we hypothesized that lower mRNA expression and higher methylation of IL-10 in BD subjects is distinguishable from those of normal ones. Owing to this, we investigated the level of interleukin-10 methylation and its gene expression in both healthy and BD patients.

2 | MATERIALS AND METHODS

2.1 | Patients and healthy controls study population

All subjects presented their written informed agreement for this study, and the study protocol was permitted by the ethics committee in Tabriz University of Medical Sciences, Tabriz, Iran (Permit Number: TBZMED.REC.1394.640). The study group consisted of 47 Iranian patients with BD (29 [61.7%] men and 18 [38.3%] women, range 16-60 years) and 61 healthy candidates. The analysis of BD was based on the international study group criteria for BD.[26] Features of the patients were evaluated at the time of the diagnosis and have been summarized in Table 2. Patients with BD were employed at the Connective Tissue Research Diseases Center of Tabriz University of Medical Sciences. The control group was composed of 61 age, gender, and ethnically matched healthy individuals (59% men vs 41% women) without any

TABLE 1 PCR primers and product size

	Sequence of primer	Product size	Tm
IL-10 for gene expression	GTTGAGCTGTTTTCCCTGA TGAAGTGGTTGGGGAATGAG	169	54
b-actin	GGTGAAGGTGACAGCAGT TGGGGTGGCTTTTAGGAT	154	55
IL-10 for medip	TGTCCCTTAAAGCCGAATGTAG CCTCCTCCTCAGAGTCAAGTTA	114	56

clinical or laboratory signs of autoimmune or inflammatory diseases.

2.2 | DNA, RNA extraction, and RT-PCR

Peripheral blood mononuclear cells (PBMCs) were prepared from EDTA blood tubes by Ficoll (Lymphodex, Inno-Train, Germany) density-gradient centrifugation and immediately stored at −80°C until use. Genomic DNA samples of BD and healthy controls were extracted by using the rapid genomic DNA extraction (RGDE) method[27] from the peripheral blood collected in tubes containing EDTA. Total RNA was extracted from the PBMCs according to the protocol of TRIzol (Invitrogen, San Diego, CA), followed by a reverse transcription using the reverse transcription reagent kit (Thermo Fisher Scientific, Waltham, MA). Furthermore, purity and concentration of total RNA were estimated by nanodrop ND1000 and at 260–280 nm purity of the RNAs were assessed. The entirety of total RNA was showed by gel electrophoresis of the individual samples on a 1% agarose gel.

2.3 | Primer design

IL-10 gene sequence and data about the promoter were picked up from The National Center for Biotechnology Information (NCBI), eukaryotic promoter database (EPD), and Ensembl (http://asia.ensembl.org/) databases. For IL-10 mRNA sequence, the primer pairs were designed using OLIGO7 Software, (Molecular Biology Insights, Inc., Cascade, CO). (Table 1) Also, IL-10 gene promoter CpG islands were anticipated with eukaryotic promoter database (EPD). A pair of primers was designed using the PrimerQuest tool to amplify CpG islands of transcription start site upstream (Figure 1).

TABLE 2 Program the qPCR for the measurement of methylation rate

Cycles	Temperature	Time	Cycle number
Activation	95°C	2 min	1
Denaturation	95°C	5 min	
Cycles	95°C	10 s	40
	54°C	20 s	
	72°C	10 s	
Final extension	72°C	2 min	1

2.4 | Real-time PCR

Peripheral blood mononuclear cells (PBMCs) were extracted from EDTA blood tubes by Ficoll (Lymphodex, Inno-Train, Germany) density-gradient centrifugation. Total RNA was extracted from the PBMCs using TRIzol (Invitrogen), followed by a reverse transcription using the reverse transcription reagent kit (Thermo Fisher Scientific), and then, the expression of IL-10 was measured by the MIC real-time instrument (Bio Molecular Systems, Australia). The following sequences of the sense and antisense primers of IL-10 were used: forward 5′-GTTGAGCTGTTTTCCCTGA-3′ and reverse 5′-TGAAGTGGTTGGGGAATGAG-3′. β-actin was chosen as the internal reference for expression and copy number variation detection and its expression was evaluated by the following primers: forward 5′-GGTGAAGGTGA-CAGCAGT-3′ and reverse 5′-TGGGGTGGCTTTTAG-GAT-3′. Relative expression levels of IL-10 were calculated using the ΔΔCt formula. All tests were performed in three biological repeats.[28]

2.5 | Methylated DNA immunoprecipitation assessment

IL-10 gene promoter CpG islands were predicted with eukaryotic promoter database (EPD). A pair of primers was designed using the Primer Quest Tool and methMarker (PREMIER Biosoft, CA) to amplify CpG islands of TSS (transcription start site) upstream (Figure 1). Methylated DNA immunoprecipitation (MeDIP) was carried out using EpiQuik™ MeDIP Ultra Kit (Epigentek, Farmingdale, NY). This Kit contains all components essential for a successful MeDIP procedure using the DNA extracted from PBMCs, such as a methylated DNA (mDNA) control, an unmethylated DNA (unDNA) control, and control primers that can be used with the control DNA to demonstrate the enrichment efficacy and specificity for methylated DNA. The extracted DNA is

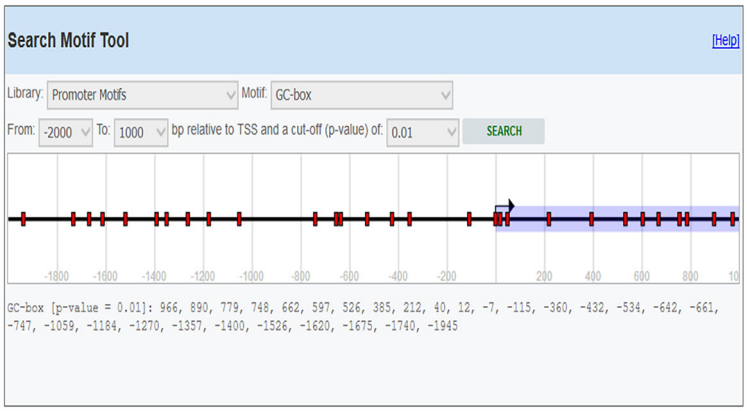

FIGURE 1 CpG islands in IL-10 promoter. CpG site and island around transcription start site (TSS) were anticipated by EPD

sonicated to produce random fragments ranging in size from 200 to 800 bp using the BANDELIN sonicator (UVV:3200, Germany) for 15 cycles of 20 s on/20 s off. Fragment size was confirmed by electrophoresis on a 1.5% agarose gel (Figure 2). Then, 1 µg of DNA is used for the following MeDIP enrichment. This method was carried out by 5 µg of fragmented genomic DNA diluted to 400 µL in TE buffer and the DNA denatured at 95°C for 5 min, followed by immediate cooling on ice for 5 min.

Immunoprecipitation stage of 5-mC enriched DNA fragments is then processed at 4°C for 2 h using 1 µL of monoclonal antibody against 5-methylcytosine (Epigentek, Farmingdale, NY) for the sample and 1 µL of normal mouse IgG as the negative control in a final volume of 100 mL antibody buffer. Further, MeDIP reaction buffers were prepared, including MeDIP buffer, sample DNA, control DNA, blocker solution, 5-mC Ab, and non-immune IgG reagents. QPCR amplification was carried out using 1 µL of eluted DNA in a 20 µL PCR reaction along with primer sets. The assays were performed in three replicates and relative methylation fold change was measured for each sample with the Fold enrichment (FE%) method which is referred to below. Detailed qPCR cycles can be found in Table 2.

The FE% of IL-10 fragments was calculated using a formula described in the manufacture protocol of this kit, using a ratio of amplification efficiency of the MeDIP sample over that of the non-immune IgG:

$$FE\% = 2^{(\text{IgG CT}-\text{Sample CT})} \times 100\%$$

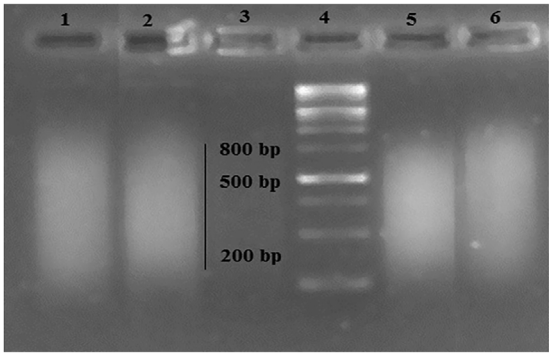

FIGURE 2 DNA shearing in gel electrophoresis. Lane [4] 100 bp ladder, the extracted DNA is sonicated and loaded in lanes (1, 2, 5, and 6)

2.6 | Enzyme-linked immunosorbent assay (ELISA)

Serum samples were individually collected from all cases and controls, plasma separated by Ficoll (Lymphodex, Inno—Train, Germany) density-gradient centrifugation and immediately stored at −80°C until use. Serum levels of IL-10 were measured with the ELISA kit that is specifically designed for these IL-10 cytokines (Catalog No. EK0416, BOSTER), according to the manufacturer's instructions. The intensity of the developed color was measured by reading the optical absorbance at 450 nm using a microplate reader (SunriseTM, Tecan Group Ltd. Männedorf, Switzerland). The ELISA reader-controlling software (Softmax) managed the digital data of raw absorbance values into a standard curve, from which the IL-10 concentration of the samples was derived. Results were expressed as a pictogram of cytokine per milliliter plasma (pg/mL).

2.7 | Statistical analysis

Statistical analysis was performed using SPSS software version 17.0 (SPSS, Chicago, IL). Normal distributions were tested with the Kolmogorov-Smirnov test with Lilliefors correction. Quantitative data were presented as

mean ± standard deviation (SD). The differences in mRNA and serum levels of IL-10 between the control and BD groups were evaluated by Mann-Whitney *U* test. *P*-value < 0.05 was considered as a significant difference.

3 | RESULTS

3.1 | Subject characteristics

Demographic and clinical characteristics of contributors exist in Table 3. The patient group consisted of 29 males and 18 females, with a mean age of 38.02 ± 10.25 years. The control subjects included 37 males and 24 females and had a mean age of 37.4 ± 8.5 years. No significant difference was observed between the age of the patients with BD and controls ($P > 0.05$).

3.2 | Real-time quantitative PCR for IL-10 gene

In order to compare the level of interleukin-10 (IL-10) gene expression in two groups of BD and healthy subjects, an independent *T*-test was adopted, taking into consideration that the data obtained by employing the Kolmogorov-Smirnov test was normal (*P* value >0.05). The obtained results were indicative of a significant difference between two groups (*P* value <0.05). As we expected, the level of gene expression showed a reduction in BD individuals group in comparison with the healthy group (Figure 3). In addition, we analyzed the relationship between gene expression and clinical features in the patient groups. As the results show, the gene expression level was significantly different in age, genital ulcers, and the severity of the disease. We see that the expression of IL-10 gene decreased in people older than 45 years than the ones less than 45 years of age (*P* value <0.05). Interleukin-10 gene expression also significantly increased in individuals with genital ulcers (*P* value <0.05). Finally, the expression level of IL-10 was significantly lower in people with severe diseases than those without a severe disease status (*P* value <0.05) (Table 3).

3.3 | Plasma levels of IL-10 in BD patients

In order to compare the serum level of interleukin-10 (IL-10) in two groups of BD and healthy individuals, an independent *T*-test was adopted, taking into consideration that the data obtained by employing the Kolmogorov-Smirnov test was normal (*P* value >0.05). The results revealed that similar to serum level, there was a statistical difference between both the groups in terms of serum level of interleukin-10 protein (*P* value <0.001). In line with our expectations, the serum level of IL-10 showed a reduction in the BD individuals group in comparison with the healthy group (Table 4, Figure 4).

3.4 | Methylation status and mRNA expression

We evaluated the association between the methylation pattern of the *IL-10* gene and mRNA expression. The mRNA (1.36 ± 0.21 versus 1.83 ± 0.69, $P < 0.05$) and serum level (28.62 ± 8.7 pg/mL vs 73.26 ± 9.2 pg/mL, $P < 0.001$) of IL-10 was significantly lower in the patients with BD than the healthy groups. In addition, in order to compare the methylation status of interleukin-10 (IL-10) in two groups of BD and healthy individuals, an independent *t*-test was adopted, taking into consideration that the data obtained by employing the Kolmogorov-Smirnov test was normal (*P* value >0.05). The results showed that there was a statistical difference between both the groups in terms of the methylation rate of interleukin-10 promoter DNA (*P* value <0.001). In line with our expectations, the methylation ratio of IL-10 revealed an increase in the BD individuals group in comparison with the healthy group (Figure 5).

Furthermore, we analyzed the relationship between the methylation level and clinical features in the patient groups. As the results show, the methylation levels were significantly different in the age groups and the severity of the disease. We see that the methylation level of IL-10 increases in people older than 45 years than those less than 45 years of age (*P* value <0.05). In addition, the methylation level of IL-10 was significantly higher in people with severe diseases than those without a severe disease status (*P* value <0.05) (Table 3).

4 | DISCUSSION

Interleukine-10 is an immune-regulatory cytokine with anti-autoinflammatory roles that plays an essential role at non-specific and acquired immune system responses.[29] Through its B cell-stimulating abilities, IL-10 participates in various stages of the B cells development, including the differentiation, activation, and survival.[30] So, it has been related to autoinflammatory diseases, such as Behçet's disease (BD).[19,30] IL-10 expression loss is common in many types of tumors and the increase of it causes to autoimmune diseases.[31,32] In addition, the expression of IL-10 within bacterial infection has been revealed to inhibit the production of autoinflammatory factors and support in the progress of Th2 immune responses. In fact, IL-10-defective mice progress with autoinflammatory diseases that are related to prominent increases in IL-12 production.[33,34]

The IL-10 gene displays significant polymorphism in the promoter site that associates with transcription. DNA methylation as a major epigenetic modification is characterized by adding a methyl group from S-adenosylmethionine to the fifth carbon of cytosine rings within CpG dinucleotides. It is clear that hypomethylation of DNA can lead to activating the transcription factors of genes; whereas hypermethylation mostly results in transcriptional gene repression.[19] New

TABLE 3 Clinical profile of patients with IL-10 gene expression and its methylation

Characteristics and clinical features expression	Frequency	Change fold of IL-10 expression (mean ± SD)	*P*-value	Methylation level of IL-10 expression (mean ± SD)	*P*-value
Age					
<45	31 (63%)	1.64 ± 0.27	**0.019**	0.68 ± 0.09	**<0.001**
≥45	14 (28%	1.26 ± 0.13		0.8 ± 0.08	
Gender					
Male	(29) 61.7%	1.53 ± 0.36	0.329	0.70 ± 0.11	0.83
female	(18) 38.3%	1.77 ± 0.79		0.69 ± 0.94	
HLA-B5-					
Positive	17 (33%)	1.41 ± 0.31	0.525	0.79 ± 0.12	0.52
Negative	11 (21%)	1.39 ± 0.15		0.76 ± 0.98	
HLA-B51					
Positive	8 (15%)	1.53 ± 0.3	0.463	0.76 ± 0.11	0.69
Negative	7 (13%)	1.41 ± 0.2		0.78 ± 0.12	
HLA-B27					
Positive	3 (6%)	1.42 ± 0.2	0.95	0.67 ± 0.1	0.72
Negative	23 (44%)	1.44 ± 0.5		0.77 ± 0.1	
Oral aphtha					
Positive	45 (96%)	1.43 ± 0.12	0.204	0.69 ± 0.05	0.29
Negative	2 (4%)	1.36 ± 0.21		0.77 ± 0.1	
Genital ulcer					
Positive	23 (49%)	1.48 ± 0.28	**0.008**	0.74 ± 0.11	0.06
Negative	24 (51%)	1.27 ± 0.16		0.79 ± 0.08	
Arthritis					
Positive	9 (19%)	1.33 ± 0.21	0.291	0.74 ± 0.11	0.05
Negative	38 (81%)	1.47 ± 0.22		0.77 ± 0.10	
Sever B.D					
Positive	30 (64%)	1.2 ± 0.07	**<001**	0.82 ± 0.08	**<0.001**
Negative	17 (36%)	1.5 ± 0.28		0.72 ± 0.09	
Severe eye involvement					
Positive	11 (22%)	1.35 ± 0.36	0.88	0.73 ± 0.10	0.28
Negative	36 (78%)	1.38 ± 0.25		0.78 ± 0.09	
EN					
Positive	39 (84.5%)	1.36 ± 0.19	0.999	0.82 ± 0.04	0.11
Negative	8 (15.5%)	1.37 ± 0.21		0.75 ± 0.11	
Phlebitis					
Positive	6 (11%)	1.33 ± 0.13	0.687	0.74 ± 0.12	0.58
Negative	41 (89%)	1.37 ± 0.22		0.77 ± 0.10	
Ocular					
No eye involvement	11 (23%)	1.38 ± 0.23	0.814	0.77 ± 0.09	0.98
One eye activity	15 (33%)	1.41 ± 0.27		0.77 ± 0.10	
bilateral activity	21 (44%)	1.31 ± 0.13		0.77 ± 0.10	
Cataract					
Positive	9 (19%)	1.29 ± 0.11	0.393	0.74 ± 0.12	0.39
Negative	36 (74%)	1.38 ± 0.22		0.77 ± 0.09	

(Continues)

TABLE 3 (Continued)

Characteristics and clinical features expression	Frequency	Change fold of IL-10 expression (mean ± SD)	*P*-value	Methylation level of IL-10 expression (mean ± SD)	*P*-value
Vision loss					
One eye	6 (13%)	1.27 ± 0.21	0.449	0.79 ± 0.11	0.65
No eye	38 (77%)	1.38 ± 0.21		0.77 ± 0.09	

As shown in the table, items that have a statistically significant difference are shown as Bold. IL-10: interleukin-10, SD, standard deviation; HLA, Human leukocyte antigen; BD, Behçet's disease.

researches have uncovered that the methylation pattern of CpG regions is correlated to cytokine expression and their serum levels.[13] So, the range of methylation at CpG region relates with the rate of cytokine production. In addition, the level of methylation at CpG sites has been revealed to be associated to cells' differentiation.[35]

The promoter status of IL-10 has numerous GC-rich sequences, which the Sp-family of transcription factors especially interacted with. Some papers have recognized that LPS-induced Sp1 and Sp3 factors can interact with these motif regions in the IL-10 promoter.[36,37] Remarkably, both the p38 and ERK MAPK signaling pathways have been associated to support Sp1 to the IL-10 promoter region.[38] It is now clear that several controlling pathways are associated in IL-10 regulation. The proximal IL-10's promoter site has many CpG islands and several transcription factors binding sites, such as CCAAT-enhancer-binding proteins (C/EBP-β), CREB-binding protein (CBP), and cAMP response elementary binding protein (CREB) that elevate the expression of certain genes, such as IL-10 via an interaction with its promoter.[39] Many studies mentioned that certain signaling pathways, such as cAMP/CREB could increase the IL-10 production in monocyte cells, and some other cytokines like IFN-γ inhibited IL-10 synergize by regulating the CREB/AP-1 signaling pathway.[39] Moreover, recently it has been established that BD inflammatory lesions involve mainly neutrophils, CD4+ T cells, and cytotoxic cells. IL-10 inhibites the proliferative responses of CD4+ human T cell clones and reduces inflammatory signaling.

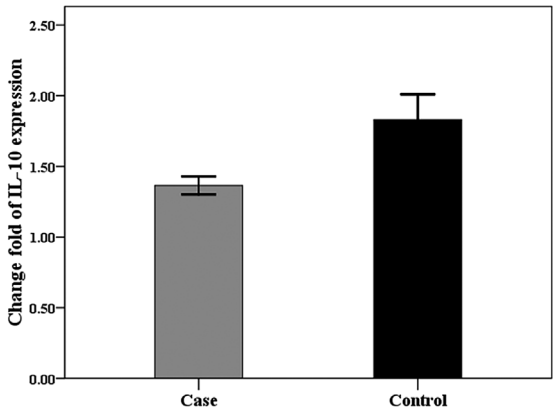

FIGURE 3 Fold change of IL-10 expression. Regarding the average changes in the expression of the IL-10 gene in the patients and the healthy groups, the amount of it is comparable to that of the healthy group in the patient group, which indicates that IL-10 gene expression was reduced among the patients in the patient group.

The hypermethylation of CpG sites, such as a promoter, is a key cause for gene low expression in diseases like autoimmune diseases and other diseases, such as cancer.[19,40,41] Therefore, to discover the correlation between IL-10 methylation status and gene expression in the pathogenesis of BD, IL-10 mRNA, protein expression, and methylation status were evaluated in the peripheral blood mononuclear cells (PBMC) of 51 BD patients and 63 healthy groups by reverse transcriptase-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and methylation DNA immune precipitation (MeDIP), respectively.

According to the results of this study, it was found that there is a negative relationship between the rate of gene expression and serum IL-10 level with the amount of methylation of this gene; thus, the patient has hypermethylation, and the gene expression level is lower in these individuals.These findings suggest that DNA methylation may be a key mechanism of IL-10 gene inactivation in BD and IL-10 hypermethylation might be involved in the occurance and development of this disease. The same results apply in the relationship between the amount of gene expression, its methylation, and clinical features; thus, their gene expression is reduced in patients with high severity (severe BD), followed by an increase in their methylation, whereas the results are exactly the opposite for people with a lower disease severity. Generally our findings suggest that demethylation treatment are able to regulate the function of IL-10 and inhibit BD disease progression.

Fu et al. investigated the methylation status of IL-10 gene at the promoter region in PBMC cells of rheumatoid patients.

TABLE 4 Plasma levels of IL-10 in BD patients

	BD (mean ± SD)	Control (mean ± SD)	*P* value
Serum level of IL-10 (pg/mL)	28.62 ± 8.7	73.26 ± 9.2	<001

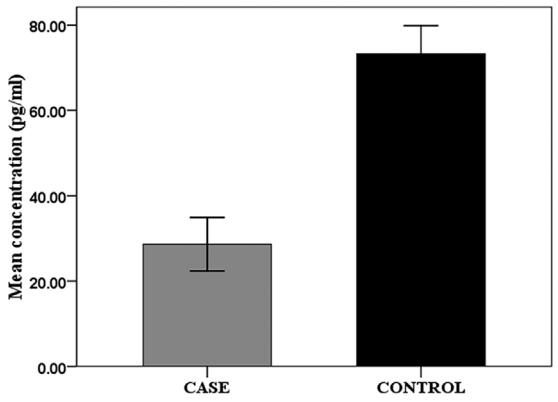

FIGURE 4 Mean serum level of IL-10. As shown in the chart, the serum levels of interleukin-10 have a significantly statistical difference in the patient and control groups, which is lower in the patient group than in the healthy group

They estimated the correlation between IL-10 promoter methylation and the regulation of gene expression in the pathophysiology of RA. The results of this study showed that the level of methylation in patients was significantly higher than in healthy subjects. It was also found that there was a negative relationship between the amount of methylation and the gene expression of IL-10 (42). Dong et al. evaluated the CpG methylation status of the IL-10 promoter and detected two slightly demethylated CpG sites in the promoter in IL–10 + versus IL-10–T-helper cells. They determined that there was no association between the methylation level and the expression of IL-10.[25] In contrast, our results revealed a significant correlation between the methylation pattern and the expression of IL-10 gene.

These results recommend that the level of methylation in the promoter region may be a key mechanism IL-10 gene regulation in BD and IL-10 CpG island hypermethylation might be involved in the amount and progress of BD.

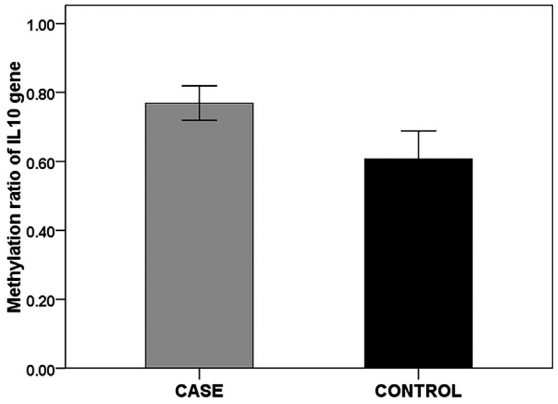

FIGURE 5 Fold change of IL-10 methylation. As shown in the chart, the level of interleukin-10 methylation in the patient group is significantly higher than the healthy group

5 | CONCLUSION

With regarding to the results of expression and methylation of interleukin-10 in this study, methylation might be one of the regulatory mechanisms involved in regulating gene expression. Considering reducing the amount of IL-10 gene expression thereafter has increased its methylation rate in patients (hypermethylation), while increasing the amount of IL-10 gene expression has decreased its methylation rate (hypomethylation). In addition, the same results apply to the relationship between the amount of gene expression, its methylation, and clinical features such that in patients with high severity, gene expression is reduced, followed by an increase in methylation; in the case of low-severity patients, these results are the opposite.

ACKNOWLEDGMENTS

This work was supported by grant from faculty of advanced medical science and department of Connective Tissue Disease, Tabriz University of Medical Sciences, Iran.

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How to cite this article: Alipour S, Nouri M, Khbbazi A, et al. Hypermethylation of IL-10 gene is responsible for its low mRNA expression in Behçet's disease. *J Cell Biochem*. 2018;1–9. https://doi.org/10.1002/jcb.26809