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Original Study

Alteration of LncRNAs Expression Level in Blood of New Case and Medicated Behcet Patients as a Prognostic Biomarker

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Abstract

Background: Behcet's disease is a complex systemic inflammatory vasculitis marked by recurrent oral and genital ulcers, and uveitis. This study aims to evaluate the expression levels of long non-coding RNAs (LncRNAs) such as *IFNG-AS1* and *AC007278.2*, along with the *IL18* and *IL18R1* genes, in the blood of active Behcet's disease patients compared to HCs. Given the elevated levels of inflammatory cytokines in these patients, exploring the role of LncRNAs could provide insights into their involvement in the disease's pathogenesis and inflammatory response.

Methods: This case-control study involved 40 Behcet's disease patients (20 medicated, 20 new cases) and 40 HCs. 2.5 ml venous blood of all subject were collected, and RNA extracted by using cDNA synthesis kit. Evaluation of genes expression level through qRT-PCR to analyze the expression levels of specific genes associated with inflammation, providing insights into Behçet's disease pathology

Results: Elevated expression of *IL-18*, *IL-18R1*, Lnc-*AC007278.2*, and Lnc-*IFNG-AS1* showed in new case patients compare to medicated patients and HCs. ROC curve analysis demonstrated high diagnostic efficacy for these biomarkers, particularly *IL18R1*, indicating their potential for accurate patient identification.

Conclusion: This study highlights the potential of LncRNAs *IFNG-AS1* and *IL18* as non-invasive biomarkers for Behcet's disease, offering insights into disease pathology and enhancing diagnostic accuracy, especially in new cases.

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1. INTRODUCTION

Behcet's disease (BD) is a systemic inflammatory vasculitis of complicated etiology that is associated with recurrent oral ulcers, genital ulcers, ocular and skin lesions, as well as manifestations such as vascular, gastrointestinal, and CNS inflammation [1]. The three main symptoms of this disease include recurrent oral ulcers, genital ulcers, and recurrent uveitis [2]. This disease correlates with significant mortality, particularly in young male patients. Involvement of large vessels, neurological, digestive, and cardiac problems are among the main causes [3]. The most common time of onset of the disease is in the third or fourth decade of life, but it can also occur at a young age and in adults. The pathergy skin test is a diagnostic hallmark of BD [4]. Although its exact etiology is not yet fully understood, recent studies have shown that genetic factors play a crucial role in developing this disease. Its highest prevalence is along the Silk Road, including countries such as Iran, Turkey, China, and Japan [5]. The concentrations of inflammatory cytokines such as IL-6, TNFα, and IL-1 are significantly increased in Behçet's patients compared to healthy controls. These cytokines, along with chemokines and other molecules, lead to increased recruitment and stimulation of inflammatory cells [6]. T Helper lymphocytes, cytotoxicity T lymphocytes, Tγδ cells, and natural killer cells play an important role in causing inflammation in Behçet's disease, leading to the recruitment and stimulation of more neutrophils and macrophages, which are directly involved in the pathogenesis of Behcet's disease [7]. Genetics and heritability significantly contribute to disease development and individual susceptibility. Recent studies have shown that genetic factors, such as HLA-B, strongly contribute to the development of the disease [8]. IL-18 is one of the pro-inflammatory cytokines of the IL-1 family, which was previously known as interferon-gamma-inducing factor, and plays a very important role in the T Helper-1 lymphocyte response. Thus, the induction of interferon-gamma secretion from T lymphocytes, and the increasing activity of NK cells are the main roles of IL-18 [9]. IL-18 and IL18R1 genes are located in chromosomal region 2q12.1. The protein encoded by IL18R1 is a functional subunit of the IL-18 receptor, which plays an important role in cellular immunity and inflammation [10]. Recent studies have proven the complex relationship between long non-coding RNAs (LncRNAs) and immune system functions, particularly in the context of inflammatory processes and autoimmune diseases [11], including rheumatoid arthritis (RA) [12], systemic lupus erythematosus (SLE) [13], polymyositis/dermatomyositis, inflammatory bowel disease, and multiple sclerosis (MS) [14], indicating their possible role in the development of these diseases. Additionally, abnormal LncRNA expression has been found to influence the secretion of crucial cytokines, such as IL-6 and TNF-α, which are essential mediators in the inflammatory processes associated with various autoimmune diseases [15]. For instance, IFNG-AS1 (also known as Tmevpg1 or LincR-Ifng-3'AS), an antisense LncRNA adjacent to the IFN-γ gene on chromosome 12, regulates IFN-γ expression in cis [16]. Given this background, the present study aims to investigate the expression levels of LncRNAs (IFNG-AS1 and AC007278.2) and immune-related genes (IL18 and IL18R1) in the blood of active BD patients compared to healthy control (HCs).

2. MATERIALS AND METHOD

2.1. Study Participants

This case-control study enrolled 80 participants, including 40 BD patients (20 on medication and 20 newly diagnosed) and 40 age- and gender-matched HCs. Participants were consecutively enrolled from Behcet's disease specialty clinic at Imam Reza Hospital (affiliated with Tabriz University of Medical Sciences) between October 2022 and December 2024. Diagnosis adhered to the International Criteria for Behcet's Disease (ICBD) and required participants to be at least 18 years old. Exclusion criteria for both patient and control groups included pregnancy, coexisting autoimmune diseases, chronic inflammatory conditions, malignancies, and recurrent infections, as well as liver or kidney diseases. The study was approved by the local Ethics Committee (Ethics code: IR.IAU.TABRIZ.1403.203) and followed the Helsinki Declaration's ethical guidelines. Informed consent was secured from all participants. The Behcet's Disease Current Activity Form (BDCAF) was utilized to evaluate disease activity, with active BD defined as BDCAF ≥1 and inactive BD as BDCAF <1.

2.2. Sampling

2.5 mL of venous blood was collected from all participants using clot-free and EDTA blood collection tubes.

2.3. RNA Extraction and cDNA Synthesis

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples utilizing Ficoll density gradient centrifugation (Ficoll-Lymphodex Innotrain, Germany), Total RNA was extracted and stored at −70°C. RNA concentration and purity were measured with a Nanodrop

spectrophotometer, and integrity was verified by 1% agarose gel electrophoresis. Following this, Complementary DNA (cDNA) was synthesized in accordance with the manufacturer's instructions using a cDNA synthesis kit.

2.4. Primer Design and Real-Time PCR

Primers for the genes under investigation were designed utilizing the NCBI Primer-BLAST tool, with their sequences provided in **Table 1**. The quantification of gene expression levels in all participants was performed using the Master Mix (Amplicon, Denmark). Each 10-µL reaction mixture contained: 5 µL of master mix, 0.25 µL of forward primer, 0.25 µL of reverse primer, 0.5 µL of cDNA, and 4 µL of RNAase-free water. The cycling reactions were performed on the StepOnePlus Real-Time PCR System (Applied Biosystems) under the following parameters: a 10-minute denaturation step at 95°C, succeeded by 45 amplification cycles, each consisting of 15 seconds at 95°C, 30 seconds at 58-62°C, and 20 seconds at 72°C.

2.5. Statistical Analysis

The fold change in gene expression for all samples was calculated using the $2^{-\Delta\Delta Ct}$ method. Differences in gene expression among the three groups were analyzed by one-way ANOVA with Bonferroni post hoc correction. GAPDH served as the housekeeping gene for normalizing all reaction data. The Shapiro-Wilk test was employed to assess the data's normal distribution. Bivariate Pearson correlation analysis was performed to evaluate: correlations between the expression levels of all target genes, and associations between gene expression and patients' demographic data. All analysis using the SPSS version 16. All figures are depicted by using Graph Pad Prism 8 software.

3. RESULTS

3.1. Participant Characteristics: Demographic, Clinical, and Laboratory Profiles

This study included 20 medicated patients with BD, 20 newly diagnosed active BD cases, and 40 sex and age-matched HC. **Table 2** illustrates the demographic and clinical profiles of the study participants. No significant differences were observed in age and gender between the patient and control groups ($p > 0.05$).

3.2. Expression Levels of Studied Genes

The expression level of IL18 was significantly elevated in the new case-patients group compared to the medicated patients' group and HCs (fold change [FC]: 9.7 vs. medicated, p=0.0004; 13.03 vs. HCs, p<0.0001) and the expression level of this gene not significantly altered in between medicated and HCs. IL18R1 expression was significantly increased in new cases compared to HCs (FC: 7.2, p<0.0001). Conversely, the expression of IL18R1 was decreased considerably in the medicated group compared to new cases (FC: -5.5, p<0.0001) but non-significantly higher expression than HCs (FC: 1.7, p=0.22). The expression levels of IFNG-AS1 were significantly elevated in the new case group (FC: 5.3, p=0.001) compared to medicated and HCs (FC: 11.8, p<0.0001). Finally, AC007278.2 showed the following: increased expression in new cases compared to HCs (FC: 6, p<0.0001); significant reduction in treated patients vs. new cases (FC: -6.65, p<0.0001) (**Table 3**, **Figure 1**).

3.3. ROC Curve Analysis

In this study, the diagnostic performance of four biomarkers IL18, IL18R1, IFNG-AS1, and AC007278.2 was evaluated. The results obtained from the ROC curve analysis demonstrate the high efficacy of these biomarkers in differentiating new case patients from HCs. IL18 exhibited a cutoff value of 10.75, with an AUC of 93%, sensitivity of 92.5%, and specificity of 85%.

IL18R1 showed the good protentional among the other genes, with a cutoff of 10.6 and an AUC of 98%. It achieved a sensitivity of 85% and a specificity of 95%, highlighting its high accuracy in distinguishing patients from HCs.

LncRNA IFNG-AS1 had a cutoff of 11.8 and an AUC of 97%, sensitivity of 95% and specificity of 92.5%, making it another reliable biomarker for diagnosing patients.

LncRNA AC007278.2 with a cutoff of 9.95 and an AUC of 97%, also provided strong results with a sensitivity of 90% and specificity of 95%.

Finally, the combined analysis of these biomarkers (**Figure 2, panel E**) yielded an AUC of 97%, sensitivity of 96%, and specificity of 97%. These results indicate that the diagnostic performance improves significantly when combining two LncRNA biomarkers (**Table 4**, **Figure 2**).

3.4. Correlation Matrix Analysis

A correlation matrix demonstrated the relationships between studied genes in new case patients, medicated patients, and healthy controls (HCs). In new case patients, IFNG-AS1 showed positive correlations with IL18 ($r = 0.81$, $p = 0.03$) and IL18R1 ($r = 0.63$, $p = 0.04$), while AC007278.2 correlated positively with IL18 ($r = 0.77$, $p = 0.002$) and IL18R1 ($r = 0.56$, $p = 0.02$). Among medicated

Table 1. List of primer sequences.

Gene	F&R	Sequence 5'-3'	Tm (C°)
IFNG-AS1	F	ACA TAC TTC CAC CAG AGA	56
	R	TTC CAC AAC ACT ATC AAC T	
AC007278.2	F	ATC ATC TGT ATG CTG TCT AAC	64
	R	AAC CAT ATA ATG AGG CTG TC	
IL-18	F	GCTGCTGAACCAGTAGAAGACA	60
	R	TAGAGGCCGATTTCCTTGGT	
IL-18R1	F	GAG AAA CAT TTT GGG TAT AAG TTA TG	60
	R	CTC TAT CAG TGA GTG GAT TTC	
GAPDH	F	CAT TGC CCT CAA CGA CCA CTT T	60
	R	GGG TCT CTC TCT TCC TCT TGT GCT	

Table 2. Demographic, clinical and laboratory characteristics of participants.

	Medicated Patients (N=20)	New Case Patients (N=20)	Healthy Control (N=40)	P-value
Demographic characteristics				
Age (mean ± SD), years	39.02 ± 9.33	41.78 ± 10.26	40.9 ± 8.5	0.680
Male (%)	15 (75)	13 (65)	20 (50)	0.623
Clinical manifestations				
Oral ulcer (%)	1 (5)	17 (85)	-	-
Skin lesions (%)	0	16(80)	-	-
Positive Pathergy (%)	0	19 (95)	-	-
Uveitis (%)	0	18 (90)	-	-
Arthralgia/Arthritis (%)	2 (10)	11 (55)	-	-
Genital ulcer (%)	0	16 (80)	-	-
CNS involvement	0	5 (25)	-	-
Vasculitis (%)	0	7 (35)	-	-
GI involvement (%)	0	6 (30)	-	-
HLA-B5 (%)	12 (60)	18 (90)	-	-
HLA-B51 (%)	15 (75)	17 (85)	-	-
BDCAF, median (IQR)	1.3 (0.2)	9.21 (5.77)	-	-
Medications				
Colchicine (%)	17 (85)	-	-	-
Prednisolone (%)	19 (95)	-	-	-
Azathioprine (%)	16 (80)	-	-	-
Methotrexate (%)	14 (70)	-	-	-
TNF inhibitors (%)	7 (35)	-	-	-
Cyclosporine (%)	8 (40)	-	-	-

SD, standard deviation; GI, gastrointestinal; CNS, central nervous system; IQR, interquartile range; BDCAF, Behcet's disease current activity form.

patients, IFNG-AS1 had a positive correlation with IL18 (r = 0.59, p = 0.005) and with IL18R1 (r = 0.19, p = 0.01), whereas AC007278.2 showed positive correlations with IL18 (r = 0.11, p = 0.01) and IL18R1 (r = 0.9, p = 0.02). In HCs, IFNG-AS1 exhibited significant positive correlations with IL18 (r = 0.23, p = 0.04) and IL18R1 (r = 0.39, p = 0.03); however, AC007278.2 showed no significant correlations with either IL18 or IL18R1 (**Table 5**). We conducted a correlation analysis to determine the relationship between the clinical, laboratory data and expression levels of genes in patients' group. The **Table 4** presents Pearson correlation coefficients (r) and P-values for various demographical factors in relation to four genes (IL18, IL18R1, AC007278.2, IFNG-AS1). The r value reflects both the magnitude and orientation of the linear association, while the p-value indicates the statistical significance of the correlation.

Table 3. Alteration of genes expression between studied groups.

		New case Patients Compare to HCs	Medicated Patients Compare to HCs	New case Patients Compare to medicated patients
IL18	FC	13.3	3.35	9.7
	P-value	<0.0001	0.49	0.0004
IL18R1	FC	7.28	1.72	5.55
	P-value	<0.0001	0.22	<0.0001
IFNG-AS1	FC	11.88	6.58	5.3
	P-value	<0.0001	<0.0001	0.001
AC007278.2	FC	5.99	0.65	6.65
	P-value	<0.0001	0.99	<0.0001

FC, Fold Change; HCs, healthy controls.

Table 4. ROC curve characteristics and sensitivity and specificity based on a cut-off value.

Genes	Specificity	Sensitivity	AUC	Cut off*	P-value
IFNG-AS1	92%	95%	97%	11.8	<0.0001
AC007278.2	95%	90%	97%	9.95	<0.0001
Combination	97%	96%	97%	10.01	<0.0001

*Cut-off is based on maximizing the sum of sensitivity and specificity; AUC: area under curve.

Table 5. Correlation of LncRNA/Protein-coding genes in new case and Medicated patients and HCs.

		IL18			IL18R1		
		New Case Patients	Medicated Patients	HCs	New Case Patients	Medicated Patients	HCs
IFNG-AS1	R	0.81	0.59	0.23	0.63	0.19	0.39
	P-value	0.03	0.005	0.04	0.04	0.01	0.03
AC007278.2	R	0.77	0.11	0.13	0.56	0. 9	0.55
	P-value	0.002	0.01	0. 4	0.02	0.02	0.09

HCs, healthy controls.

Gender showed statistically insignificant correlations with all genes. Age was positively correlated with IL18 (r = 0.29, p = 0.02) and IL18R1 (r = 0.13, p = 0.048). Weight demonstrated positive correlations with IL18 (r = 0.235, p =0.0318), IL18R1 (r = 0.29, p = 0.04), and AC007278.2 (r = 0.69, p = 0.003). BMI showed a positive correlation with IL18 (r = 0.178, p = 0.045), IL18R1 (r = 0.217, p = 0.035), and AC007278.2 (r = 0.375, p = 0.010). Genital ulcer exhibited positive correlations with IL18 (r = 0.185, p = 0.035), IL18R1 (r = 0.086, p = 0.018), and AC007278.2 (r = 0.165, p = 0.0487). Skin involvement was positively correlated with IL18 (r = 0.239, p = 0.011) and with IFNG-AS1 (r = 0.241, p = 0.006). Oral ulcer had positive correlations with IL18 (r = 0.20, p = 0.034), IL18R1 (r = 0.313, p = 0.017), and AC007278.2 (r = 0.310, p = 0.018). Uveitis showed positive correlations with IL18 (r = 0.191, p = 0.041), IL18R1 (r = 0.183, p = 0.044), AC007278.2 (r = 0.168, p = 0.048), and IFNG-AS1 (r = 0.297, p = 0.004). Arthralgia demonstrated a positive correlation with AC007278.2 (r = 0.291, p = 0.021), while arthritis was positively correlated with IL18R1 (r = 0.235, p = 0.032), AC007278.2 (r = 0.244, p = 0.029), and IFNG-AS1 (r = 0.248, p = 0.029). CNS involvement showed a positive correlation with IL18 (r = 0.13, p = 0.04), IL18R1 (r = 0.73, p = 0.03), and AC007278.2 (r = 0.388, p = 0.01). Gastrointestinal involvement was positively correlated with IL18 (r = 0.168, p = 0.047), IL18R1 (r = 0.121, p = 0.011), AC007278.2 (r = 0.267, p = 0.025), and IFNG-AS1 (r = 0.65, p = 0.008). Finally, BDCAF showed positive correlations with IL18 (r = 0.44, p = 0.005), IL18R1 (r = 0.88, p = 0.012), and AC007278.2 (r = 0.467, p = 0.038). We also analyzed the correlation of gene expression and medication use in medicated patients. Based on these analyses, colchicine showed a negative correlation with IL18 (r = -0.362, p = 0.022), IL18R1 (r = -0.093, p = 0.050), AC007278.2 (r = -0.229, p = 0.055), and IFNG-AS1 (r = -0.496, p = 0.001). Prednisolone showed a negative correlation with IL18 (r = -0.191, p = 0.038), IL18R1 (r = -0.195, p = 0.027),

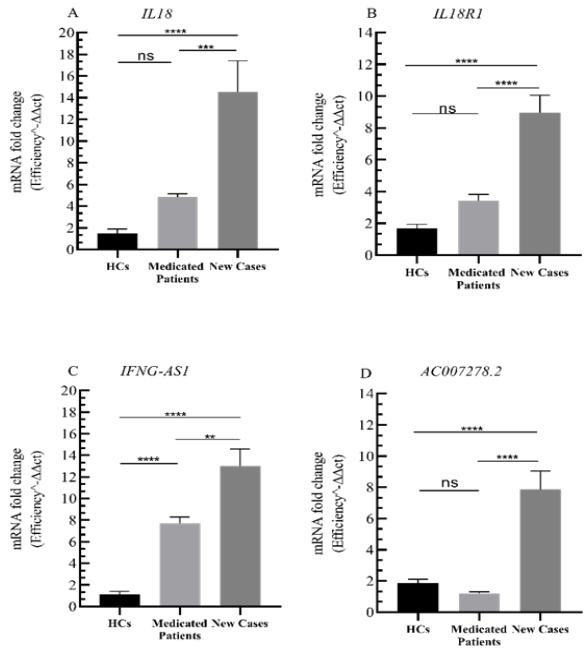

Figure 1. The expression level of genes between patients and HCs.

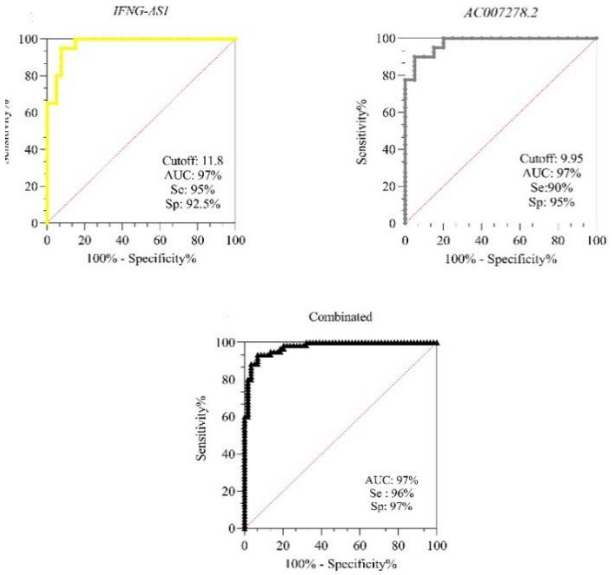

Figure 2. The roc curve analysis genes between new case patients and HCs.

AC007278.2 (r = -0.033, p = 0.040), and IFNG-AS1 (r = -0.334, p = 0.035). Methotrexate showed a negative correlation with IL18 (r = -0.469, p = 0.002), IL18R1 (r = -0.135, p = 0.008), AC007278.2 (r = -0.209, p = 0.010), and IFNG-AS1 (r = -0.636, p = 0.001). Azathioprine showed a negative correlation with IL18 (r = -0.364, p = 0.021), IL18R1 (r = -0.200, p = 0.015), AC007278.2 (r = -0.163, p = 0.016), and IFNG-AS1 (r = -0.613, p = 0.001). Cyclophosphamide showed a negative correlation with IL18 (r = -0.319, p = 0.048), IL18R1 (r = -0.150, p = 0.031), AC007278.2 (r = -0.517, p = 0.001), and IFNG-AS1 (r = -0.677, p = 0.001).

4. DISCUSSION

Studies have shown that BD is an autoimmune disease in which genetics plays an important role in its pathogenesis, and this disease has been proposed as a polygenic disease. For example, the HLA-B51 allele, which is located in the histocompatibility complex on chromosome 6, is one of the common alleles in Turkey and Japan, which is commonly associated with the disease [17]. Despite the knowledge of the genetic pathways associated with this disease, there is still no specific and definitive marker available for diagnosis, which poses additional challenges in the diagnosis and treatment of this disease. Long non-coding RNAs may be present in biological fluids such as blood and urine, and this feature could make them interesting as non-invasive biomarkers, and they can have high sensitivity and specificity in identifying pathological conditions [18]. Based on recent studies, the lncRNAs as biomarkers have a high potential for the diagnosis and prognosis of diseases. This area of research is still developing, but it could contribute to a better understanding of disease mechanisms and the provision of more effective treatments [19]. IFNG-AS1 is an antisense lncRNA located near the interferon-gamma gene locus. Its expression is regulated by the transcription factor T-bet. Increased expression of this gene is directly related to increased IFNG production from T-helper1 cells, and recent studies have shown that IFNG-AS1 expression induces the expression of inflammatory factors such as NFκB [20]. In the present study, the expression level of the IFNG-AS1 gene in the blood of new case patients was significantly increased by about 11 folds compared to healthy control subjects, while the expression level of it in medicated patients showed a significant decrease compared to new case patients. Also, in a study by Peng et al., it was shown that the expression levels of T-Bet, IFNG-AS1, and IFNG genes were increased in the blood of RA patients compared to healthy individuals [16]. In a study by Javidi et al., they showed that increased IFNG-AS1 expression causes increased IFNG expression in treatment-naïve schizophrenia patients, and there is a direct relationship between them [21]. Therefore, many studies have proven that increased IFNG-AS1 expression increases the interferon-gamma production, also the present study is one of the first studies to evaluate the expression of these genes in patients with a history of drug use and new case. In this study, we demonstrated that the expression level of gene IL18 in new case patients has significantly increased in the new case group compared to HCs, also, the expression level

of IL18 in medicated patients is significantly decreased compared to new case patients. IL18 induces both innate and adaptive immune activity and, by affecting the function of T-helper 1 cells, macrophages, natural killer cells, B cells, and dendritic cells, causes the production of interferon gamma so it is known as an IFNG secretion-inducing factor. According to several studies, the expression level of IL18 significantly increased in lupus, psoriasis, MS [22], CVD [23], and CKD [24] patients. In a study of Gangemi et al. on psoriasis, they revealed that the expression level of IL18 in patients was significantly higher than in HCs [25]. A study by Calvani et al, showed that the expression levels of IL18 were increased in patients' biopsies compared to HCs the expression level of the IL18 gene was also increased in affected patients compared to HCs. Finally, by using ELISA, they showed that the serum level of IL18 in patients was significantly elevated compare to HCs [26]. Therefore according to recent studies the increased expression of IL18 is directly related to onset and the severity of the disease. Also we showed that, the expression level of the IL18R gene significantly increased in new case patients compare to HCs, so that the expression level of the IL18R on the same side of IL18 in new case patients, and using DMRAD medication cause the decreasing of IL18 and IL18R in medicated patients.

In this study, we also investigated the role of lncRNA AC007278.2 in regulating the IL18R gene. The lncRNA AC007278.2 are located in intronic region of IL18R [27]. In this study, we showed that the expression level of AC007278.2 increased significantly in new case patients compared to HCs, and in medicated patients, due to DMARD consumption, its expression significantly decreased compared to new case patients. In a study by You et al. showed that the expression level of AC007278.2 in lupus patients was significantly increased compare to HCs [27]. In study of Javidi and et al, revealed that the expression level of AC007278.2 was increased in schizophrenia new case patients compared to medicated patients [21]. The results of the ROC curve analysis demonstrate that the selected biomarkers exhibit excellent diagnostic performance. Specifically, the P-values for all evaluated markers are less than 0.0001, indicating highly significant statistical results. The cut-off points identified for each biomarker are 11.8 for IFNG-AS1, 9.95 for AC007278.2, and 10.01 for the combined marker panel. The Area Under the Curve (AUC) values are all remarkably high at approximately 97%, reflecting excellent discrimination ability between the conditions studied. In terms of sensitivity, IFNG-AS1 shows a rate of 95%, while AC007278.2 achieves a sensitivity of 90%. The combined marker panel demonstrates the highest sensitivity at 96%, suggesting it is slightly more effective in correctly identifying true positive cases. Regarding specificity, IFNG-AS1 presents a value of 92%, AC007278.2 has a specificity of 95%, and the combined panel exhibits the highest specificity at 97%. These findings collectively indicate that the combined gene signature offers superior overall diagnostic accuracy, with maximum sensitivity and specificity, supported by an AUC of 97%, making it a promising candidate for clinical diagnostic applications. Also, we used Pearson correlation analysis, which reveals interesting insights into the relationships between gene expression and various demographic and clinical parameters in the study population. This analysis sheds light on how factors such as age, weight, BMI, and clinical manifestations correlate with gene expression, particularly focusing on IL18, IL18R1, AC007278.2, and IFNG-AS1. A significant positive correlation was observed between IL18, IL18R1and age. This may indicate a cumulative effect of age on inflammatory processes mediated by these cytokines. Conversely, gender did not show any significant correlation, pointing to potential uniformity across sexes in the studied sample regarding genetic expression. The presence of clinical features such as genital ulcer, skin involvement, oral ulcer, uveitis, and others displayed positive correlations with various genes. Uveitis had a positive correlation with IL18 and IL18R1. Gastrointestinal complications showed strong correlations with IL18 and IFNG.AS1, further emphasizing an inflammatory role that IL18 may play in various systemic manifestations. The correlations involving AC007278.2 frequently reached significant levels, suggesting it might be a potential biomarker for assessing inflammatory conditions in this cohort. For example, a particularly strong correlation was noted with weight and BMI, hinting at a possible linkage between BMI and inflammatory responses. The analysis included the effects of various drug treatments like colchicine, prednisolone, methotrexate, azathioprine, and cyclophosphamide, which influenced the gene expression negatively: IL18 was noted to have negative correlations post-treatment with all drugs, indicating a significant downregulation of IL18 levels, which could suggest effective modulation of the inflammatory response in patients: Methotrexate showed a strong negative correlation ($r = -0.469$, $p = 0.002$), indicating that this therapy might robustly diminish IL18 expression. Likewise, cyclophosphamide also exhibited a very strong negative correlation with IFNG-AS1, suggesting that increased IL18 is intricately tied to reductions in this gene, potentially mechanistically linking immune responses to treatment regimens. IFNG-AS1, across most treatments, demonstrated a very strong negative

correlation with IL18, highlighting a consistent inverse relationship that may underscore the regulatory pathways at play during treatment. For example, after Azathioprine treatment, the correlation was (r = -0.613, p = 0.001), indicating significant downregulation as treatment progresses.

5. CONCLUSION

In summary, this study highlights the significant roles of lncRNAs and cytokine-related genes, particularly IFNG-AS1, IL18, IL18R, and AC007278.2, in the pathogenesis and diagnosis of autoimmune diseases such as Behçet's Disease. The findings demonstrate that these genes are differentially expressed in patients compared to healthy controls, with elevated levels correlating with disease activity and severity. The strong diagnostic performance of the combined gene panel, evidenced by an AUC of approximately 97% and high sensitivity and specificity, underscores their potential as reliable non-invasive biomarkers. Additionally, correlation analyses suggest that gene expression levels are influenced by demographic factors, clinical features, and treatment regimens, indicating their relevance in disease monitoring and therapeutic response. Overall, these molecular markers offer a significant understanding of the fundamental inflammatory mechanisms and could facilitate more precise diagnostics and personalized treatment strategies in autoimmune conditions.

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Conflict of interest

The authors declare that they have no conflict of interest

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Ethical statement

The study protocol was reviewed and approved by the Research Ethics Committee of the Islamic Azad University, Tabriz Branch (IR.IAU.TABRIZ.REC.1403.203).

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