RESEARCH ARTICLE

Proinflammatory cytokine gene polymorphisms in Behcet's disease

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ABSTRACT. Behçet's disease (BD) is a chronic, systemic disease, characterized by oral and genital lesions, and ocular inflammation. There is evidence indicating altered levels of proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) in patients with BD. This study involved 150 patients with BD and 140 healthy controls, and investigated the role of proinflammatory cytokine gene polymorphisms in the disease. The frequency of the TNF-α (-238) G/G genotype was significantly higher in the patient group, compared to the controls ($p < 0.001$), whilst the G/A genotype was significantly lower in the patients with BD ($p < 0.001$). Patients with BD showed a significant increase in the TNF-α (- 308, - 238) GG haplotype ($p < 0.001$), whilst there was a significant decrease in the GA haplotype ($p < 0.001$). The heterozygous, IL-6 (- 174) C/G genotype ($p = 0.005$), and the IL-6 (- 174, nt565) haplotype CG ($p < 0.001$), were significantly decreased in the patient group. The increased production of proinflammatory cytokines in BD could be a consequence of specific, cytokine gene polymorphisms. Particular genotypes and haplotypes in TNF-α were over-represented in BD, which may, in turn, predispose individuals to this disease.

Keywords: Behcet's disease, gene polymorphisms, interleukin-6, tumor necrosis factor alpha

Behçet's disease (BD) is a chronic, systemic disease, characterized by oral and genital lesions, and ocular inflammation. It is thought to have an autoimmune origin on the basis of the vasculitic nature of the disease [1].

The role of proinflammatory cytokines in BD is the subject of debate. There have been a number of reports indicating altered levels of interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) in patients with BD [2-5]. Production of proinflammatory cytokines could be affected by genetic polymorphisms within promoter regions of cytokine genes. Thus the genetic predisposition of individuals to produce high or low amounts of a particular cytokine may affect the disease. Recently, some studies have shown a role for proinflammatory cytokine gene polymorphisms in the susceptibility to some diseases [6-10].

A number of recent studies, particularly in the Turkish population, have focused on IL-6 and TNF-α gene polymorphisms in BD; however, such a study has not been undertaken in the Iranian population [11-22]. This study was designed to look for a number of proinflammatory cytokine gene polymorphisms in Iranian patients with BD.

DONORS AND METHODS

This study involved 150 patients with BD, according to the International Study Group (ISG) criteria [23], who were referred to the Rheumatology Research Center (Tehran, Iran), and included140 healthy controls, who attended the Iranian Blood Transfusion Center [24]. This study was approved by the local Ethics Committee and informed consent was obtained from all subjects.

All cytokine typing were performed on genomic DNA using the polymerase chain reaction with the sequence-specific primers (PCR-SSP) assay. The PCR-SSP kit used was the Heidelberg cytokine gene polymorphism SSP kit (Heidelberg University, Heidelberg, Germany).

The method has been described previously in detail [24]. Briefly, amplification was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. The allele and genotype frequencies of the proinflammatory cytokine genes, including IL-1α (T/C - 889), IL-1β (C/T −511, T/C + 3962),

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IL-1R (C/T pstI 1970), IL-1RA (T/C mspaI 11100), TNF-α (G/A - 308, G/A - 238), and IL-6 (G/C - 174, G/A nt565) were determined.

Data analysis was performed using the Epi Info statistical software (version 6.2, World Health Organization, Geneva, Switzerland). Allele frequencies were estimated by direct gene counting. Allele and genotype frequencies were compared using the chi-square test. The odds ratio and 95% confidence intervals (CI) were calculated for each allele/genotype/haplotype in both patient and control groups. A P-value of less than 0.05 was considered significant. The Bonferroni correction method was used to adjust for multiple comparisons.

RESULTS

Demographic data, clinical characteristics and laboratory data of patients with BD are presented in the *tables 1* and *2*. TNF-α - 308 and - 238 genotype and allele frequencies in patients with BD and healthy controls are shown in *table 3*. Allele G at the - 238 of the TNF-α gene showed a significant increase in BD patients (93% *vs* 79%, $p < 0.001$). The frequency of the G/G genotype was significantly higher in the patient group (86% *vs* 58%, $p < 0.001$) with an odds ratio of 4.4 (95% CI: 2.4-8.2), while the G/A genotype was significantly lower in the patients with BD (14% *vs* 42%, $p < 0.001$). *Table 4* shows the haplotype frequency of TNF-α - 308 and - 238. Patients with BD showed a significant increase in the TNF-α GG haplotype (83% *vs* 64%, $p < 0.001$), whilst there was a significant decrease in the GA haplotype (7% *vs* 21%, $p < 0.001$).

The heterozygous C/G genotype at -174 of IL-6 was found in 67% for the control subjects versus 50% of patients with BD, which was significantly lower ($p = 0.005$, OR: 0.49, 95%CI: 0.3-0.8) *(table 3)*. The frequency of the CG haplotype in patients with BD was also significantly lower than that found in the controls (9% *vs* 20%, $p < 0.001$) *(table 4)*.

Table 1
Clinical characteristics of patients with Behcet's disease

Characteristics	
Male/Female ratio	90/60
Mean age (years) (SD)	26.3 (9.94)
Age at onset (years)	*Number (percentage)*
< 16	21 (14)
17-39	119 (79.3)
> 40	10 (6.7)
Type of involvement	*Number (percentage)*
Oral aphthosis	150 (100)
Genital aphthosis	99 (66)
Skin	101 (67.3)
Ocular	109 (72.7)
Joint	48 (32)
Central nervous system	16 (10.7)
Vascular	9 (6)
Gastrointestinal	9 (6)
Epidydimitis (men)	7 (7.8)

Table 2
Laboratory findings for patients with Behcet's disease

	Total test performed	**Number (percentage)**
Positive pathergy test	150	93 (62)
High ESR (> 20)	150	68 (45.3)
Abnormal urine	149	13 (8.7)
VDRL/RPR	148	1 (0.7)
HLA B5	150	84 (56)
HLA B51	32	19 (59.4)
HLA B27	145	6 (5.5)

DISCUSSION

In this study, an increased frequency of the G allele at - 238 TNF-α was found in patients with BD, whereas the A allele was significantly decreased at same position. This is in contrast to the previous study by Ahmad *et al.*, in the UK [25], that showed the TNF-α (- 238) A allele had a higher frequency (18%) in BD patients compared to healthy individuals (9.3%). Also, in the studies by Ates *et al.* [14] and Dilek *et al.* [16] in a Turkish population, and Storz *et al.* [21] in a Turkish and a German population, no significant difference for the TNF-α (- 238) A or G allele was found between patients and healthy controls. TNF-α is a proinflammatory cytokine that has been implicated in the pathogenesis of autoimmune diseases. Polymorphic alleles at positions - 308 (G to A) and - 238 (G to A) have been reported in the promoter region of the TNF- α gene [26-29]. There are conflicting reports on effect of TNF-α (- 238) on the expression level of TNF-α. It seems that the TNF-α (- 238) G/G genotype corresponds to lower levels of TNF-α in peripheral blood. Grove *et al.* [30] suggested that the TNFA-A allele is associated with increased TNF-α expression, whilst other investigators have reported that the mean level of TNF-α production was not significantly different between TNFA-G/G homozygous and TNFA-A/G heterozygous individuals in different disease entities [27, 31-33]. Kaluza *et al.* [34] reported the TNF (- 238) A allele to have significantly decreased transcriptional activity, and peripheral blood mononuclear cells carrying this allele produced significantly less TNF-α after stimulation with T cell mitogens. Our results indicate that the TNF (- 238) G/G genotype contributes to the development of BD. Therefore, it could be suggested that the apparent increase in the G/G genotype is responsible for the high-producer phenotype reflecting the increase in the level of serum and intraocular TNF-α in BD patients [5]. The meta-analysis by Touma *et al.* [22] on TNF polymorphisms in patients with BD in various ethnic groups, which has recently been published, showed that - 238A (OR = 1.51; 95% CI = 1.12-2.04) had a significant association with BD. It should be noted that there was no significant difference in the TNF-α promoter region at - 308 G to A polymorphism between Iranian patients with BD and healthy controls, which is in agreement with the results of the meta-analysis by Touma *et al.* [22]. Although the study by Park *et al.* [20] in Korean patients

Table 3
Comparisons of allele and genotype frequencies between patients with BD and controls

Cytokine	Position	Alleles/ Genotypes	Patients with BD (n = 147) n (%)	Controls (n = 140) n (%)	P-value	Odds ratio (95% CI)
IL-1α	- 889	C	204 (69.4)	186 (68.4)	0.867	-
		T	90 (30.6)	86 (31.6)		
		CC	73 (49.7)	62 (45.6)	0.571	-
		TC	58 (39.4)	62 (45.6)	0.356	-
		TT	16 (10.9)	12 (8.8)	0.703	-
IL-1β	- 511	C	161 (54.8)	154 (55.4)	0.946	-
		T	133 (45.2)	124 (44.6)		
		CC	38 (25.9)	36 (25.8)	0.900	-
		TC	85 (57.8)	82 (59)	0.936	-
		TT	24 (16.3)	21 (15.2)	0.904	-
IL-1β	+ 3962	C	203 (69.5)	198 (70.7)	0.826	-
		T	89 (30.5)	82 (29.3)		
		CC	65 (44.5)	70 (50)	0.418	-
		TC	73 (50)	58 (41.4)	0.182	-
		TT	8 (5.5)	12 (8.6)	0.428	-
IL-1R	Pst-I 1970	C	201 (68.4)	174 (62.1)	0.139	-
		T	93 (31.6)	106 (44.2)		
		CC	63 (42.9)	54 (38.6)	0.536	-
		TC	75 (51)	66 (47.1)	0.590	-
		TT	9 (6.1)	20 (14.3)	0.036*	0.39 (0.16-0.95)
IL-1RA	Mspa-I 11100	C	75 (25.5)	64 (22.9)	0.519	-
		T	219 (74.5)	216 (77.1)		
		CC	0 (0)	4 (2.9)	0.055	-
		CT	75 (51)	56 (40)	0.079	-
		TT	72 (49)	80 (57.1)	0.205	-
TNF-α	-308	A	28 (9.5)	39 (14.2)	0.108	-
		G	266 (90.5)	235 (85.8)		
		AA	0(0)	0 (0)	-	-
		AG	28 (19)	39 (28.5)	0.084	-
		GG	119 (81)	98 (71.5)	0.084	
TNF-α	- 238	A	21 (7.1)	59 (21.5)	< 0.001*	0.28 (0.16-0.49)
		G	273 (92.9)	215 (78.5)	< 0.001*	3.57 (2.04-6.27)
		AA	0 (0)	1 (0.7)	0.482	-
		GA	21 (14.3)	57 (41.6)	< 0.001*	0.23 (0.13-0.43)
		GG	126 (85.7)	79 (57.7)	< 0.001*	4.41 (2.40-8.15)
IL-6	- 174	C	91 (31)	101 (36.3)	0.203	-
		G	203 (69)	177 (63.7)		
		CC	9 (6.1)	4 (2.9)	0.302	-
		CG	73 (49.7)	93 (66.9)	0.005*	0.49 (0.29-0.81)
		GG	65 (44.2)	42 (30.2)	0.020	1.83 (1.09-3.07)
IL-6	nt565	A	66 (22.4)	50 (18)	0.221	-
		G	228 (77.6)	228 (82)		
		AA	2 (1.4)	4 (2.9)	0.437	-
		GA	62 (42.2)	42 (30.2)	0.048	1.68 (1.00-2.83)
		GG	83 (56.4)	93 (66.9)	0.090	-

* Significant at 5% level, adjusted for the Bonferroni multiple testing correction.

Table 4
Comparisons of haplotype frequencies of TNF-α and IL-6 between patients with BD and controls

Cytokine	Haplotype	Patients with BD (n = 147) n (%)	Controls (140 subjects) n (%)	P-value	Odds ratio (95% CI)
TNF-α	GG	245 (83.3)	176 (64.2)	< 0.001*	2.78 (1.84-4.21)
(-308,-238)	AG	28 (9.6)	39 (14.2)	0.108	-
	GA	21 (7.1)	59 (21.5)	< 0.001*	0.28 (0.16-0.49)
IL-6	GG	200 (68)	173 (62.2)	0.172	-
(-174,nt565)	CG	27 (9.2)	55 (19.8)	< 0.001*	0.41 (0.24-0.69)
	CA	66 (22.5)	46 (16.6)	0.094	-
	GA	1 (0.3)	4 (1.4)	0.205	-

* Significant at 5% level, adjusted for the Bonferroni multiple testing correction.

with BD showed that the TNFA - 308 G allele was associated with an increased risk of BD, the results of other previous studies were compatible with our study, reporting no significant difference in the distribution of TNF-α (- 308) allele and genotype frequencies [17-19, 35].

It has been shown that IL-6 can play a role in the pathogenesis of BD. IL-6 concentrations in culture supernatants from patients with active BD were significantly high, and accordingly, IL-6 gene expression was enhanced [4]. Active BD was characterized by a higher level of IL-6 compared that found in BD in remission [36]. In this study, an increased frequency of the G/G genotype and a significant decreased frequency of the C/G genotype (- 174) and CG haplotype were found in patients with BD. This is in contrast with study by and Dilek *et al.* in a Turkish population [16]. Studies of IL-6 (- 174) polymorphisms in Korean BD patients showed no genetic association [15]. There is a marked difference between genotype frequencies in healthy Korean people and our findings in healthy Iranian subjects, indicating ethnic variations. A large body of evidence indicates an increased serum level of IL-6 in patients with active BD [2-4, 36-39], especially in patients with ocular involvement [40] where it acts as a local inflammatory mediator in pathological processes. The genotype G/G corresponds to a higher production of IL-6 [41-43], or at least is part of the haplotypes of IL-6-promoter associated with increased transcription level [44].

Although there was no association between IL-1 and BD, a specific genotype and haplotype (GG) in TNF-α were over-represented in BD, which may have predisposed individuals to this disease. However, further studies are needed to test the association between such polymorphisms and the immunological phenomena seen in this group of patients.

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