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CLINICAL STUDY

Interleukin 10 and transforming growth factor beta 1 gene polymorphisms in juvenile idiopathic arthritis

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ABSTRACT

OBJECTIVES: The aim of this study is to identify the associations between interleukin 10 (IL-10) and transforming growth factor beta 1 (TGF-β1) gene polymorphisms and individual susceptibility to juvenile idiopathic arthritis (JIA) in a group of Iranian patients.

BACKGROUND: Cytokine genes, including IL-10 and TGF-β1, are known to play important roles in the pathogenesis of JIA.

METHODS: Using polymerase chain reaction with sequence-specific primers method, the frequency of alleles, genotypes and haplotypes of IL-10 (positions -1082, -819, -592) and TGF-β1 (codon 10, codon 25) single-nucleotide polymorphisms (SNPs) were investigated in 55 patients with JIA as a case group and compared with 140 healthy unrelated controls.

RESULTS: The G allele was significantly less frequent at TGF-β1 codon 25 in patients with JIA than in the controls ($p < 0.01$). The frequency of CT genotype at TGF-β1 codon 10 was found to be higher in healthy individuals in comparison with that in patients group ($p = 0.04$). We observed no differences in the frequency of alleles, genotypes and haplotypes of IL-10 gene between the groups of patients and controls.

CONCLUSIONS: Considering the low frequency of existence of TGF-β1 G allele at codon 25 as well as TGF-β1 CT genotype at codon 10 in patients with JIA, it seems that these cytokine gene polymorphisms could play role as the protective factors against JIA *(Tab. 2, Ref. 42)*. Text in PDF *www.elis.sk*

KEY WORDS: interleukin 10, transforming growth factor beta 1, single-nucleotide polymorphism, juvenile idiopathic arthritis, children.

Introduction

Juvenile idiopathic arthritis (JIA) belongs to a group of disorders known as chronic inflammatory arthopathies. It is the most common rheumatic disorder with an incidence of 1/10,000 children under the age of 16 years (1). It is widely accepted that JIA is a heterogeneous group of complex diseases with both environmental and genetic factors contributing to its initiation and progression (2). Although the major histocompatibility locus is a well-known genetic factor involved in the etiopathogenesis of JIA, it does not account for the overall genetic contribution to this disease (3). To date, immunogenetic analyses in JIA have focused on the aforementioned gene, while fewer studies have assessed non-HLA gene variants such as cytokines gene polymorphisms, that have been related to the susceptibility to JIA (4, 5).

Interleukin 10 (IL-10), a potent immunoregulatory cytokine, is mainly released by T helper 2 (Th2) cells, macrophages and B cells, and acts as a suppressor of the production of a number of proinflammatory cytokines such as interleukin 6 (IL-6), interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α) (6, 7). IL-10 has been recommended as a stimulatory factor for B lymphocytes, thymocytes as well as mast cells. Decreased IL-10 secretion has been documented in autoimmune disorders, including collagen-induced arthritis, psoriasis and rheumatoid arthritis (RA) (8, 9).

Transforming growth factor beta 1 (TGF-β1) is a regulator of the immune response in JIA. This cytokine exerts both anti-inflammatory and pro-inflammatory effects, depending on several, mostly unveiled, factors. TGF-β1 induces the differentiation of leukocytes, notwithstanding the fact that it inhibits the multiplication of T cells and the activation of monocytes and macrophages (10, 11).

Contribution of certain cytokines single-nucleotide polymorphisms (SNPs) to a number of immunological diseases has been

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documented (12–18), while our comprehension of JIA is limited due to the paucity of investigations in this area. To the best of our knowledge, this is the first study evaluating possible associations of SNPs in anti-inflammatory cytokines genes with JIA susceptibility in Iranian population.

In the present study, the associations between SNPs in IL-10 at positions -1082, -819 and -592 together with TGF-β1 at codon 10 and codon 25 and JIA were evaluated in Iranian patients.

Patients and methods

Subjects

We recruited fifty-five patients with the clinical diagnosis of JIA from the Rheumatology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Iran and 140 healthy controls who were randomly selected from blood donors at Iranian blood transfusion organizations in the study (19). All patients met the International League Against Rheumatism (ILAR) criteria for JIA (20).

All the entrants to the study gave written informed consent and the study was approved by the Ethical Committee of Tehran University of Medical Sciences.

Genotyping

Genomic DNA was isolated from whole blood treated with EDTA by a standard "salting out" technique (21). The final preparation was kept at –20 degrees Celsius until investigation. The IL-10 (A/G at -1098, C/T at -819 and A/C at -592) as well as TGF-β1 (C/T at codon 10 and C/G at codon 25) were determined by polymerase chain reaction with sequence-specific primers (PCR-SSP assay kit, Heidelberg University) (19). Amplification of the extracted gene was performed by a Techne Flexigene thermal cycler (Roche). The following cycling conditions were used: initial denaturation at 94 °C for 2 minutes; denaturation at 94 °C for 10 seconds; annealing + extension at 65 °C for 1 minute (10 cycles); denaturation at 94 °C for 10 seconds; annealing at 61 °C for 50 seconds; and extension at 72 °C for 30 seconds (20 cycles). PCR products were visualized on a 2 % agarose gel electrophoresis. Thereafter, ultraviolet (UV) transillumination was carried out so as to take a picture for analysis and documentation.

Statistical analysis

The allele, genotype and haplotype frequencies were estimated by direct gene counting and compared by the chi square test. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each allele, genotype, and haplotype. Chi square test was used for the evaluation of the Hardy–Weinberg equilibrium. The probability value of less than 0.05 was considered significant.

Results

Allelic and genotype frequencies in Iranian patients with JIA and healthy control subjects are depicted in Table 1.

The frequency of C allele of TGF-β1 codon 25 was significantly higher in the patients category in comparison with that in healthy controls (95.5 % vs 7.6 %, $p < 0.01$). At the genotype level, we observed a lower distribution of heterozygous CT of TGF-β1 codon 10 in patients compared to that in controls (49.1 %

Tab. 1. TGF-β1 and IL-10 allele and genotype polymorphisms in Iranian patients with juvenile idiopathic arthritis and controls.

Cytokine	Position	Alleles/Genotypes	Controls (n=140) n (%)	Patients (n=55) N (%)	Odds Ratio (95% CI)	p value
TGF-β1	Codon 10	C	131 (47.5)	55 (50)	1.11 (0.69–1.76)	0.73
		T	145 (52.5)	55 (50)	0.90 (0.57–1.44)	0.73
		CC	20 (14.5)	14 (25.5)	2.01 (0.87–4.65)	0.11
		CT	**91 (65.9)**	**27 (49.1)**	**0.50 (0.25–0.99)**	**0.04**
		TT	27 (19.6)	14 (25.4)	1.40 (0.63–3.12)	0.48
TGF-β1	Codon 25	**C**	**21 (7.6)**	**105 (95.5)**	**255.00 (87.48–802.27)**	**<0.01**
		G	**255 (92.4)**	**5 (4.5)**	**0.00 (0.00–0.01)**	**<0.01**
		CC	2 (1.5)	0 (0)	0.00 (0.00–10.37)	1.00
		GC	17 (12.3)	5 (9.1)	0.71 (0.22–2.20)	0.70
		GG	119 (86.2)	50 (90.9)	1.60 (0.52–5.19)	0.52
		A	181 (64.6)	66 (68.8)	1.20 (0.71–2.04)	0.54
		G	99 (35.4)	30 (31.2)	0.83 (0.49–1.40)	0.54
IL-10	-1082	AA	53 (37.8)	20 (41.7)	1.17 (0.57–2.41)	0.77
		GA	75 (53.6)	26 (54.2)	1.02 (0.50–2.08)	0.92
		GG	12 (8.6)	2 (4.2)	0.46 (0.07–2.31)	0.52
		C	199 (71.1)	62 (63.3)	0.70 (0.42–1.17)	0.19
		T	81 (28.9)	36 (36.7)	1.43 (0.85–2.38)	0.19
IL-10	-819	CC	71 (50.7)	20 (40.8)	0.67 (0.33–1.36)	0.30
		CT	57 (40.7)	22 (44.9)	1.19 (0.58–2.41)	0.73
		TT	12 (8.6)	7 (14.3)	1.78 (0.59–5.27)	0.27
		A	81 (28.9)	36 (36.7)	1.43 (0.85–2.38)	0.19
		C	199 (71.1)	62 (63.3)	0.70 (0.42–1.17)	0.19
IL-10	-592	AA	12 (8.6)	7 (14.3)	1.78 (0.59–5.27)	0.27
		CA	57 (40.7)	22 (44.9)	1.19 (0.58–2.41)	0.73
		CC	71 (50.7)	20 (40.8)	0.67 (0.33–1.36)	0.30

Tab. 2. TGF-β1 and IL-10 haplotype polymorphisms in Iranian patients with juvenile idiopathic arthritis and controls.

Cytokine	Position	Haplotype	Controls (n=140) n (%)	Patients (n=55) n (%)	Odds Ratio (95% CI)	p value
TGF-β1	Codon10, Codon25	CG	110 (39.9)	51 (46.4)	1.30 (0.82–2.09)	0.29
		TG	145 (52.5)	54 (49.1)	0.87 (0.55–1.39)	0.62
		CC	21 (7.6)	4 (3.6)	0.46 (0.13–1.46)	0.23
		TC	0 (0)	1 (0.9)	–	0.17
IL-10	-1082, -819, -592	GCC	99 (35.4)	30 (31.3)	0.83 (0.49–1.40)	0.54
		ACC	100 (35.7)	32 (33.3)	0.90 (0.54–1.51)	0.76
		ATA	81 (28.9)	34 (35.4)	1.35 (0.80–2.27)	0.29

vs 65.9 %, p = 0.04). The allele and genotype frequencies of IL-10 at positions -1082, -819 and -592 were similar in two groups of patients and controls.

As depicted in Table 2, no significant difference was detected between the two groups, neither for GCC, ACC and ATA haplotypes at positions -1082, -819 and -592 of IL-10 gene nor for CG, TG, CC and TC haplotypes at codon 10 and codon 25 of TGF-β1 gene.

Discussion

Juvenile idiopathic arthritis (JIA) is known to be a clinically heterogeneous entity with various underlying genetic predisposing factors involved in the etiopathogenesis of this group of disorders. Among the aforesaid gene variants, the association of JIA with certain SNPs within the promoter and coding regions of a multitude of cytokines genes, including IL-10 and TGF-β1, have been examined in different ethnic groups with inconsistent results (22–24). Furthermore, promoter and coding regions polymorphisms in IL-10 and TGF-β1 genes have been correlated with altered levels of circulating IL-10 and TGF-β1. Moreover, the majority of the existing data were obtained from the association studies performed on adult patients with rheumatoid arthritis. Therefore there is a paucity of data with regard to the association of the aforementioned cytokine gene SNPs with JIA. This study aims at analyzing the association of IL-10 -1082 G/A, -819 C/T, -592 C/A) and TGF-β1 (codon 10 C/T, codon 25 C/G) polymorphisms in Iranian patients with JIA.

TGF-β1, a member of a family of growth factors, which has been detected in the synovial tissue of RA patients, exerts modulatory effects on lymphocytes, macrophages, dendritic cells, chondrocytes, fibroblasts and osteoblasts. This cytokine has been detected in both inactive and the active forms in rheumatoid joints (25). The gene encoding TGF-β1 is situated at chromosome 19q13, which comprises seven exons (26). Two cytokine SNPs located on codon 10 (T869C, Leu/Pro) and codon 25 (G915C, Arg/Pro) in the coding sequence of TGF-β1 gene, both of which have been reported to be correlated with the variations in the levels of TGF-β1, were examined in this study (27). In the present study, TGF-β1 CT genotype at codon 10 was significantly higher in healthy controls compared with that in JIA patients. On the contrary, Cinek et al did not recognize any association between TGF-β1 SNPs and JIA proneness in Czech population (28). Two meta-analysis studies carried out by Chang et al (29) and Zhang et al (30) divulged an association between TGF-β1 T869C polymorphism and RA in the people of Asian descent, but not in the people of non-Asian descent. On the other hand, our findings are not in line with the investigation performed by Hussein et al, who found the TGF-β1 T allele at codon 10 to be associated with susceptibility to RA (25). In addition, our findings revealed the decreased frequency of G allele of TGF-β1 at codon 25. On the other hand, Muñoz-Valle et al found no associations between TGF-β1 G915C polymorphism with RA (31).

IL-10, known as an immunoregulatory cytokine, exerts its antiinflammatory effect by impeding the production of proinflammatory cytokines, including TNF-α, IL-1β, and IL-8 by human polymorphonuclear leukocytes (32), IFN-γ synthesis in T cells (33), and IL-1α, IL-1β, IL-6, IL-8, IL-12, and TNF-α release from activated macrophages (34). Additionally, IL-10 appears to be effective in blocking mononuclear cell traffic into synovial tissue by downregulating intercellular adhesion molecule 1 expression by synovial cells (35, 36), mitigating inflammation in the collagen-induced arthritis animal model (37), inhibiting the action of proinflammatory cytokines IL-1α and IL-1β by enhancing the release of soluble IL-1 receptor antagonist (34), reversing cartilage degradation by mononuclear cells from RA patients in tissue culture (38), and ameliorating proinflammatory cytokine synthesis by mononuclear cells from peripheral blood, synovial cells and synovial fluid of patients with RA (38). Previous studies on patients with JIA have indicated the reduced production of IL-10 from whole blood culture (39). The gene encoding for IL-10 is situated within a highly conserved cytokine gene cluster on chromosome 1q32 (40). In the current study, we have examined 3 functional SNPs at -1082A/G (rs1800896), -819C/T (rs3021097), and -592A/C (rs1800872) of IL-10. Associations between these IL-10 gene variants and RA have been reported (23, 41). We observed no associations between the above-mentioned IL-10 gene polymorphisms with individual's susceptibility to JIA in Iranian population. Similarly, Cinek et al found no significant association between IL-10 gene variants and JIA vulnerability in Czech population (28). Contrarily, Möller et al demonstrated a significantly increased frequency of low IL-10 expressing -1082A/A alleles, medium IL-10 expressing ACC haplotype, and GTC haplotype, as well as the decreased frequency of heterozygous -1082G/A alleles, and the GCC haplotype in patients with systemic juvenile idiopathic arthritis (sJIA) in a German population (42). Fife et al also revealed a significant increased

prevalence of the low expressing IL-10 -1082 genotype in British patients with sJIA (3).

In conclusion, our results suggest that the TGF-β1 G allele at codon 25 as well as TGF-β1 CT genotype at codon 10 confers protection against JIA in Iranian population. Nevertheless, our findings should be interpreted with caution due to the small number of the patients enrolled in the study. The other limitation of our investigation that needs to be acknowledged is our constraint to measure the levels of IL-10 and TGF-β1 production and thus, our inability to analyze the influence of cytokine gene SNPs on their level of synthesis. As a result, recruitment of larger sample size in populations of different ethnicities are required to verify the roles of these polymorphisms of the IL-10 and TGF-β1 genes in the pathogenesis of RA.

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