The role of tumor necrosis factor-alpha -308 G/A and transforming growth factor-beta 1 -915 G/C polymorphisms in childhood idiopathic thrombocytopenic purpura

Çocukluk çağı idiopatik trombositopenik purpura'da TNF-α-308G/A ve TGF-β1-915G/C polimorfizmlerinin rolü

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Abstract

Objective: To increase our understanding of the etiology of idiopathic thrombocytopenic purpura (ITP) some cytokine gene polymorphisms were analyzed for susceptibility to the disease. The aim of this study was to investigate the role of tumor necrosis factor-alpha (TNF-α) -308 G/A and transforming growth factor-beta 1 (TGF-β1) –915 G/C polymorphisms in the development and clinical progression of childhood ITP.

Materials and Methods: In all, 50 pediatric patients with ITP (25 with acute ITP and 25 with chronic ITP) and 48 healthy controls were investigated via LightCycler® PCR analysis for TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms.

Results: The frequency of TNF-α -308 G/A polymorphism was 20%, 16%, and 22.9% in the acute ITP patients, chronic ITP patients, and controls, respectively (p>0.05). The frequency of TGF-β1 -915 G/C polymorphism was 16%, 8%, and 8.3% in the acute ITP patients, chronic ITP patients, and controls, respectively (p>0.05). The risk of developing ITP and clinical progression were not associated with TNF-α -308 G/A (OR: 0.738, 95% CI: 0.275-1.981, and OR: 0.762, 95% CI: 0.179-3.249) or TGF-β1 -915 G/C (OR: 1.5, 95% CI: 0.396-5.685, and OR: 0.457, 95% CI: 0.076-2.755) polymorphisms.

Conclusion: The frequency of TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms did not differ between pediatric ITP patients and healthy controls, and these polymorphisms were not associated with susceptibility to the development and clinical progression of the disease. *(Turk J Hematol 2011; 28: 170-5)*

Key words: Idiopathic thrombocytopenic purpura, childhood, TNF-α, TGF-β1, polymorphism

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Özet

Amaç: İdiopatik trombositopenik purpura (ITP)'nın etyolojisini anlayabilmek için, hastalığa yatkınlık üzerinde bazı sitokin gen polimorfizmleri incelenmiştir. Bu çalışmanın amacı çocukluk çağı ITP'sinin gelişimi ve klinik seyrinde tümör nekrozis faktör-alfa (TNF-α) –308G/A ve transforming growth faktör-beta1 (TGF-β1) -915G/C polimorfizmlerinin rolünü araştırmaktır.
Yöntem ve Gereçler: Elli ITP'li çocuk hasta (25 akut ITP, 25 kronik ITP) ve 48 sağlıklı kontrol TNF-α–308G/A ve TGF-β1–915G/C polimorfizmleri için Light Cycler PCR analizi ile araştırıldı.
Bulgular: Akut ITP, kronik ITP ve kontrollerde TNF-α–308G/A polimorfizm sıklığı sırasıyla %20, %16 ve %22.9 (p>0.05), ve TGF-β1–915G/C polimorfizm sıklığı sırasıyla %16, %8 ve %8.3 (p>0.05) idi. ITP gelişim riski ve klinik seyri ile TNF-α–308G/A (OR: 0.738, %95 Cl: 0.275-1.981 ve OR: 0.762, %95 Cl: 0.179-3.249) ve TGF-β1–915G/C (OR: 1.5, %95 Cl: 0.396-5.685 ve OR: 0.457, %95 Cl: 0.076-2.755) polimorfizmleri arasında ilişki bulunamadı.
Sonuç: Bu sonuçlar, çocukluk çağı ITP hastalarında TNF-α–308G/A ve TGF-β1–915G/C polimorfizmlerinin sıklığının sağlıklılardan farklı olmadığını göstermiş olup, bu polimorfizmlerin ITP gelişimi ve hastalığın klinik seyri için risk oluşturmadığını düşündürmektedir. *(Turk J Hematol 2011; 28: 170-5)*
Anahtar kelimeler: İdiopatik trombositopenik purpura, çocukluk çağı, TNF-α, TGF-β1, polimorfizm

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Introduction

Idiopathic thrombocytopenic purpura (ITP) is one of the most frequent causes of acquired thrombocytopenia and is characterized by destruction of autoantibody-mediated platelets. The pathophysiology of ITP is complex and includes antibodies, cytokines, antigen-presenting cells, co-stimulatory molecules, and T- and B-lymphocytes. When autoreactive B-lymphocytes produce anti-platelet antibodies, T-lymphocytes play an important role in this autoimmune process [1-3].

Children with ITP have a Th1-type cytokine pattern with elevated levels of tumor necrosis factor-alpha (TNF-α), as in most autoimmune diseases [4]. Researches have shown that polymorphisms in the TNF-α gene at position -308 and -238 affect gene transcription with increased TNF-α production [5,6]. The transcriptionally more active allele of TNF-α (allele 2 of -308) was less frequently observed in pediatric chronic ITP patients than in healthy controls [7].

Transforming growth factor-beta 1 (TGF-β1) is another multifunctional cytokine that plays a critical role in the pathogenesis of inflammatory, autoimmune, and malignant diseases. The expression of TGF-β1 is influenced by polymorphisms in the TGF-β1 gene, which may be partly relevant to certain diseases [8-10]. It was reported that some TGF-β1 polymorphisms are not associated with the development and progress of ITP [11]. The aim of the present study was to determine if TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms play a role in the development and clinical progression of ITP in children.

Materials and Methods

Study population

The study was conducted at Ankara University, Faculty of Medicine, Department of Pediatric Hematology, Pediatric Molecular Pathology and Genetics Division, and at Ankara Dışkapı Children's Health Training and Research Hospital, Department of Pediatric Hematology. In all, 50 pediatric ITP patients (25 acute ITP and 25 chronic ITP) and 48 healthy controls were enrolled in this study. Children diagnosed with acute or chronic ITP and regularly followed-up in the hospitals participating in the study were considered for inclusion.

The diagnosis of ITP was based on medical history, physical examination, and thrombocytopenia. Thrombocytopenia that resolved within 6 months of onset was defined as acute ITP and thrombocytopenia that persisted for >6 months was defined as chronic ITP. All the controls had a negative history of thrombocytopenia and ITP, and their blood samples were obtained from the DNA bank of Ankara University, Faculty of Medicine, Department of Pediatric Hematology, Pediatric Molecular Pathology and Genetics Department. Informed consent was obtained from all the patients, the controls, and their parents. The study protocol was approved by the Ankara University Faculty of Medicine Ethics Committee.

Genomic DNA analysis, and TNF-α -308 G/A and TGF-β1 -915 G/C polymorphism genotyping

Peripheral blood samples were obtained from all the participants. Blood samples were collected in

microfuge tubes containing ethylenediamine tetraacetic acid as anticoagulant. Genomic DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method.

TNF-α -308 polymorphism

Regions containing TNF-α -308 G/A polymorphisms were amplified via polymerase chain reaction (PCR) using 0.1 μg/mL concentration of 2 primer sets, according to our previous report [12].

TGF-β1 -915 polymorphism

Regions containing TGF-β1 -915 G/C polymorphisms were amplified via PCR using 0.1 μg/mL concentration of 2 primer sets. The primer sequences used for the TGF-β1 -915 promoter region were as follows:

Forward: 5'-ACC ACA CCA GCC CTG TTC GC-3';
Reverse: 5'-AGC TTG GAC AGG ATC TTG CC-3'.

PCR yielded a 224-base pair (bp) long oligonucleotide product and the results were confirmed based on 2% agarose gel electrophoresis containing 0.5 mg/mL ethidium bromide at 100 W for 45 min using an 18-μL PCR product. The gels were evaluated with a UV transilluminator imaging analyzer.

Statistical analysis

Statistical analysis was performed using SPSS v.15.0 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). The demographic features of the acute and chronic ITP patients were compared using Student's t-test and the Mann-Whitney U-test. The odds ratio and 95% confidence interval (CI), as estimates of the relative risk of allele frequency, were calculated for the entire study population. The 95% CI was calculated based on a conditional logistic regression algorithm using the maximum likelihood method.

Results

In the present study 50 ITP patients (25 acute ITP and 25 chronic ITP) and 48 controls were analyzed for TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms. The demographics of the acute and chronic ITP patients are shown in Table 1. The acute ITP patients were aged 4 months to 12 years (median age: 6 years), and the chronic ITP patients were aged 18 months to 15.5 years (median age: 9 years). The patients with chronic ITP were significantly older ($p<0.05$). Hemoglobin, leukocyte, and platelet counts in the acute and chronic ITP patients at admission did not differ significantly ($p=0.12$, 0.99, and 0.64, respectively).

In total, 5 (20%) of the 25 acute ITP patients, 4 (16%) of the 25 chronic ITP patients, and 11 (22.9%) of the 48 control subjects had TNF-α -308 G/A gene polymorphism. The frequency of TNF-α -308 G/A gene polymorphism in the acute ITP, chronic ITP, and control groups did not differ significantly ($p>0.05$). In addition, the A allele frequency did not differ significantly between the 3 groups ($p>0.05$).

The frequency of TGF-β1 -915 G/C polymorphism in the acute ITP, chronic ITP, and control groups was 16%, 8%, and 8.3%, respectively, but the difference was not significant ($p>0.05$). C allele frequency was 2-fold higher in the acute ITP patients (8%) than in the chronic ITP patients (4.1%) and controls (4%); the difference was not statistically significant ($p>0.05$). The distribution of TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms in the study and control groups are shown in Tables 2 and 3.

The risk of developing ITP was not associated with TNF-α -308 G/A (OR: 0.738, 95% CI: 0.275-1.981) or TGF-β1 -915 G/C (OR: 1.5, 95% CI: 0.396-5.685) polymorphisms. The frequency of TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms in the acute and chronic ITP patients did not differ significantly

Table 1. Patient demographics

	Acute ITP (n=25)	Chronic ITP (n=25)	p
Age (years)	5.6±3.5	8.0±3.9	0.028
Sex (M/F)	15/10	11/14	>0.05
Hemoglobin (g/dl)*	11.5 (8.0-14,9)	11.9 (6.8-14.5)	>0.05
Leukocyte (/mm^3)*	8400 (4900-17300)	8100 (5000-23900)	>0.05
Platelet (/mm^3)*	8000 (1000-25000)	8000 (1000-78000)	>0.05

*Results are expresed as median (range)

Table 2. Distribution of TNF-α-308G/A polymorphism in ITP, acute ITP, chronic ITP patients and controls

Genotype frequency	Controls n=48 n (%)	ITP patients n=50 n(%)	Acute ITP n=25 n (%)	Chronic ITP (n=25) n (%)	Fischer's exact test	OR (95% CI)
G/G	37 (77.1)	41 (82)	20 (80)	21 (84)	0.546[a]	0.738 (0.275-1.981)
G/A	11 (22.9)	9 (18)	5 (20)	4 (16)	1.000[b]	0.762 (0.179-3.249)

OR Odds ratio; CI confidence interval, [a]ITP group compared with the controls, [b]Acute ITP group compared with chronic ITP group

Table 3. Distribution of TGF-β1-915G/C polymorphism in ITP, acute ITP, chronic ITP patients and controls

Genotype frequency	Controls n=48 n (%)	ITP patients n=50 n (%)	Acute ITP n=25 n (%)	Chronic ITP n=25 n (%)	Fischer's exact test	OR (95% CI)
G/G	44 (91.7)	44 (88)	21 (84)	23 (92)	0.741[a]	1.5 (0.396-5.685)
G/C	4 (8.3)	6 (12)	4 (16)	2 (8)	0.667[b]	0.457 (0.076-2.755)

OR Odds ratio; CI confidence interval, [a]ITP group compared with the controls, [b]Acute ITP group compared with chronic ITP group

($p>0.05$), and these polymorphisms did not have an affect on chronic progression of the disease (OR: 0.762, 95% CI: 0.179-3.249 and OR: 0.457, 95% CI: 0.076-2.755, respectively).

Discussion

Although the etiology of ITP remains unclear, it is generally accepted that both environmental and genetic factors play an important role in the development of the disease [13,14]. Many recent studies have focused on the association between some cytokine gene polymorphisms and susceptibility to ITP. Studies that investigated genetic risk factors in the pathogenesis of ITP reported that polymorphisms of HLA class II antigens, FcγRIIA and FcγRIIIA, and human platelet antigen (HPA) systems were associated with the development of ITP and response to treatment [7,15-19].

TNF-α is a pleiotropic cytokine produced primarily by macrophages and T-cells, and has a range of inflammatory and immunomodulatory activity [20-22]. Polymorphisms of TNF-α promoter are associated with high levels of TNF-α and have been studied as a determinant of susceptibility to numerous diseases [12,21-25]. A study that examined inflammatory cytokines and Fcγ receptor polymorphisms in chronic childhood ITP reported that polymorphisms in 2 low-affinity FcγR genes (IIIA and IIIB) and 2 proinflammatory cytokine genes (TNF and LTA) were associated with chronic childhood ITP [7]. To the best of our knowledge the role of TNF-α -308 G/A polymorphism in the development and progression of ITP has not been previously studied.

TGF-β is a multifunctional cytokine and TGF-β1 is the most abundant isoform [26,27]. TGF-β1 gene polymorphisms that alter TGF-β1 production have been studied in predicting susceptibility to certain diseases [8,9]. A significant increase in the percentage of NK cells and elevated TGF-β1 levels have been observed in chronic ITP patients in remission [28]. Although it has been shown that TGF-β1 gene polymorphisms (509 G/C, codon 25, and codon 10) were significantly correlated with many diseases and TGF-β1 production, the only study on the role of these polymorphisms in the pathophysiology of ITP reported that they weren't correlated with the development, clinical progression, or treatment response of the disease [11,29-32].

In the present study the genotype frequency of TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms was evaluated in terms of their correlation with susceptibility to the development and progression of ITP. At the time of diagnosis it is not possible to predict the progress of the disease. To date, no prognostic factors for the development and clinical progression of the disease have been reported [33]. The results of the present study show that the frequency of TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms in the acute ITP, chronic ITP, and control groups did not differ significantly. Moreover, associations between these polymorphisms, and the development and clinical progress of ITP were not observed.

Plasma TNF-α and TGF-β1 levels in the patient and control groups were not analyzed in the present study. It is known that a substitution at the -308 position of TNF-α results in increased production of

TNF-α [6], and that polymorphisms at codon 10 and 25 of the TGF-β1 gene are involved in the upregulation of TGF-β1 secretion. Other studies failed to observe any association between serum TGF-β1 levels and these polymorphisms [34].

In conclusion, TNF-α -308 G/A and TGF-β1 -915 G/C polymorphisms did not play an important role as genetic risk factors for the development and progression of ITP. As the study and control groups were small, different results may be obtained by additional research involving larger patient populations.

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Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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