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The Associations of *IL-6, IFN-γ, TNF-α, IL-10,* and *TGF-β1* Functional Variants with Acute Myeloid Leukemia in Turkish Patients

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Aim: It has been suggested that cytokine dysregulation could be associated with pathogenesis, progression, and survival in acute myeloid leukemia (AML). The purpose of this study was to evaluate the relationship of functional single-nucleotide polymorphisms (SNPs) in cytokine gene and cytokine expression levels with AML. *Materials and Methods:* Peripheral blood samples were collected from 42 patients with AML and 85 healthy individuals. Eight SNPs in five cytokine genes, including interleukin 6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and transforming growth factor-β 1 (TGF-β1), were analyzed using the polymerase chain reaction sequence-specific primer method. *Results:* We found that the frequencies of the TNF-α (−308) GG genotype and G allele were significantly higher in the patients with AML compared to the healthy control group ($p = 0.020$ and 0.014). The AML patients had significantly lower frequencies of the CC genotype and C allele of the IL-10 (−819 SNP), the G allele of the IL-10 (−1082 SNP), the CC genotype and C allele of the IL-10 (−592 SNP), and the codon 25 GC genotype of TGF-β1, ($p = 0.024$, $p = 0.012$, $p = 0.038$, $p = 0.024$, $p = 0.012$, $p = 0.028$, respectively). However, no significant differences were found between AML and healthy control groups with respect to the distributions of genotypes in IL-6, IFN-γ, IL-10 (−1082), TGF-β1 (codon 10), and haplotypes of IL-10, TGF-β1 gene. *Conclusion:* Our results suggest that functional variants of the TNF-α, IL-10, and TGF-β1 genes may have a significant association with the etiopathogenesis of AML. Further studies with larger groups and different ethnicities are needed to determine the impact of cytokine variants on the risk of developing AML.

Introduction

Acute leukemia is classified as acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Cases with AML account for ∼33% of adolescent and 50% of all adult leukemias (Seval and Ozcan, 2015). AML, a heterogeneous and complex disease, is characterized by clonal proliferation of myeloid precursors and associated with reduced capacity of these cells to differentiate into mature cells (Ali *et al.,* 2014). Although the specific cause of this biological abnormality has not been completely defined yet, it is thought that both genetic and environmental factors play important roles in molecular pathogenesis. The factors that affect the apparent increase in the incidence of AML include aging population, toxic exposures, and previous chemotherapy and/or radiation use for the treatment of other disorders (Wang, 2014). Several genetic variants have been reported as potential risk factors for leukemia (Mutlu *et al.*, 2014; Urbanowicz *et al.*, 2010).

Cytokines, humoral immunomodulatory proteins or glycoproteins, regulate the immune responses and many other biological processes. They are produced by immune cells upon stimulation. Cytokines, getting bound to specific receptors, may upregulate activation, proliferation, and differentiation of target cells, mediate or regulate immune reactions, inhibit the growth of cells, act as cytotoxic agents, and induce or inhibit the production of other cytokines. Pro- and anti-inflammatory cytokines play a key role in identifying patients with high risk for several diseases (Holleward and Bidwell, 2006; Fei *et al.*, 2015; Winkler *et al.*, 2015). The lack of balance between pro- and anti-inflammatory cytokines hinders proper functioning of the immune system. Recent studies have found that any difference in cytokine levels (high or low) has an association with certain allelic variants of cytokine genes (Trifunović *et al.*, 2015). Several cytokine gene variants located at regulatory or promoter regions can cause various cytokine expressions (Karaoglan *et al.*, 2009).

Therefore, we hypothesize that cytokine gene variants may be associated with pathogenesis of AML. First, we investigated the relationship of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interferon-γ (IFN-γ), interleukin-10

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(IL-10), and transforming growth factor-β 1 (TGF-β1) gene functional variants with susceptibility to AML in Turkish people. Second, using the haplotype-based analytic approach, we tested whether haplotypes of IL-10 and TGF-β1 are associated with AML.

Materials and Methods

Study subjects

A total of 42 cases with primary AML (19 males and 23 females, aged between 18 and 62) were included in the research. Patients were diagnosed and their follow-ups were done in the Department of Internal Medicine Section of Hematology at Gaziantep University. AML diagnosis was made according to the World Health Organization criteria based on the increased number of myeloblasts in bone marrow or peripheral blood (Vardiman *et al.*, 2008). In addition, the control group consisted of 85 unrelated healthy subjects (40 males and 45 females; aged between 19 and 60) with similar ethnic background and geographic origin with the patients. Subjects without evidence of any personal and/or family history of cancer or other serious illness were included in the control group. Informed consent was obtained from each participant before blood sampling, and the study was approved by the local Ethical Committee of Gaziantep University.

Genotyping analysis

Genomic DNA was extracted from whole ethylenediamine tetracetate-treated blood using a salting-out procedure (Miller *et al.*, 1988). The investigated functional gene variants included IL-6 −174G/C (rs1800795), IFN-γ +874 (rs62559044), TNF-α −308 (rs1800629), IL-10-1082G/A (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872), and TGF-β1 codon 10 (rs1982073) and codon 25 (rs1800471). Single-nucleotide polymorphism (SNP) of these cytokines was investigated with the methods of polymerase chain reaction sequence-specific primer (Karaoglan *et al.*, 2009). This is a kit in which all regions are studies at the same amplification. It was a place in literature and was given as a reference to avoid redundancy.

Statistical analyses

All data were analyzed using software SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL). The statistical significance of the differences between the patient and control groups was estimated by logistic regression analysis. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. Differences in IL-6, INF-γ, TNF-α, IL10, and TGF-β1 genotype frequencies between the patient and control groups were compared with chi-square test, and Fisher's exact test was used when needed. All analyses were two-tailed, and differences were interpreted as statistically significant when $p < 0.05$.

Results

Forty-two patients with AML and 85 control individuals were analyzed. Female subjects were predominant in the patient and control group. The genotypic and allelic frequencies of the TNF-α, IL-6, IFN-γ, IL-10, and TGF-β1 functional

Table 1. Comparison of Genotype and Allele Frequencies of TNF-α, IL-6, and IFN-γ Gene Variants Between Patients with AML and Healthy Controls

	AML	*Control*			
Genotype/allele	n[a] (%)	n[b] (%)	*OR*	*95% CI*	p[c]
TNF-α (−308)					
GG[d]	41 (97.6)	71 (83.5)	8.085	1.025–63.740	**0.020**
AG[d]	1 (2.4)	12 (14.1)	6.740	0.846–53.708	0.059
AA[e]	0 (0)	2 (2.4)	1.024	0.991–1.058	1.000
A	1 (1.2)	16 (9.4)			
G	83 (98.8)	154 (90.6)	8.623	1.124–66.176	**0.014**
IL-6 (−174)					
GG[d]	21 (50)	49 (57.6)	0.735	0.350–1.543	0.452
GC[d]	20 (47.6)	31 (36.5)	0.631	0.298–1.336	0.252
CC[e]	1 (2.4)	5 (5.9)	2.563	0.290–22.664	0.663
C	22 (26.2)	41 (24.1)			
G	62 (73.8)	129 (75.9)	0.896	0.492–1.632	0.785
IFN-γ (+874)					
TT[d]	11 (26.2)	8 (9.4)	0.442	0.153–1.274	0.157
TA[f]	15 (35.7)	45 (53)	2.025	0.946–4.336	0.089
AA[e]	16 (38.1)	32 (37.6)	0.981	0.458–2.102	1.000
T	37 (44.1)	61 (35.9)			
A	47 (55.9)	109 (64.1)	0.711	0.417–1.211	0.220

The results that are statistically significant are typed in bold.
[a]n = 42.
[b]n = 85.
[c]Fisher's exact test.
[d]High gene expression.
[e]Low gene expression.
[f]Intermediate gene expression.
AML, acute myeloid leukemia; CI, confidence interval; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.

variants in patients with AML and healthy controls are shown in Tables 1 and 2. Since sample size is small, clinical parameters were not included because reliable data could not be obtained in terms of statistical significance when the groups were subdivided.

TNF-α (–308) genotype

The frequencies of the GG, GA, and AA genotypes of TNF-α variant in the AML group were 97.6%, 2.4%, and 0%, respectively. Compared with the control group, TNF-α 308 genotype GG and allele G showed a significant difference in AML patients (OR = 8.085, 95% Cl 1.025–63.740, $p = 0.020$; OR = 8.623, 95% Cl 1.124–66.176, $p = 0.014$, respectively).

IL-6 (–174) genotype

The frequencies of the genotypes of IL-6 variant were evaluated as GG (50.0%), GC (47.6%), and CC (2.4%) in the AML group. No significant difference in any genotype or allele frequency was observed between the AML patients and the controls.

Table 2. Comparison of Genotype and Allele Frequencies of TGF-β1, and IL-10 Variants Between Patients with AML and Healthy Controls

	AML	*Control*			
Genotype/allele	n[a] (%)	n[b] (%)	*OR*	*95% CI*	p[c]
TGF-β1 (codon 10)					
CC	12 (28.6)	24 (28.2)	0.984	0.434–2.232	1.000
TC	24 (57.1)	48 (56.5)	1.134	0.535–2.401	0.849
TT	6 (14.3)	13 (15.3)	1.083	0.380–3.086	1.000
T	36 (42.9)	74 (43.6)			
C	48 (57.1)	96 (56.4)	1.028	0.606–1.742	1.000
TGF-β1 (codon 25)					
GG	36 (85.6)	64 (75.3)	1.969	0.728–5.325	0.249
GC	3 (7.2)	20 (23.5)	4.000	1.116–14.340	**0.028**
CC	3 (7.2)	1 (1.2)	0.155	0.016–1.536	0.105
C	9 (10.7)	22 (12.9)			
G	73 (89.3)	148 (87.1)	1.239	0.544–2.893	0.687
TGF-β1 haplotype					
TTGG, TCGG[d]	30 (71.4)	55 (64.7)	1.364	0.610–3.046	0.549
TCGC,CCGG, TTGC[e]	9 (31.4)	26 (30.6)	1.616	0.677–3.855	0.301
CCGC, CCCC, TTCC, TCCC[f]	3 (7.2)	4 (4.7)	0.642	0.137–3.009	0.684
IL-10 (–1082)					
AA	22 (52.4)	32 (37.6)	0.549	0.260–1.159	0.130
AG	17 (40.5)	36 (42.4)	1.080	0.510–2.291	1.000
GG	3 (7.1)	17 (20)	3.250	0.896–11.794	0.073
G	23 (27.4)	70 (41.2)			
A	61 (72.6)	100 (58.8)	1.857	1.051–3.278	**0.038**
IL-10 (–819)					
CC	15 (35.7)	49 (57.6)	2.450	1.141–5.259	**0.024**
CT	21 (50)	32 (37.6)	0.604	0.286–1.275	0.251
TT	6 (14.3)	4 (4.8)	0.286	0.079–1.114	0.081
T	33 (39.3)	40 (23.5)			
C	51 (60.7)	130 (76.5)	0.476	0.271–0.835	**0.012**
IL-10 (–592)					
CC	15 (35.7)	49 (57.6)	2.450	1.141–5.259	**0.024**
CT	21 (50)	32 (37.6)	0.604	0.286–1.275	0.251
TT	6 (14.3)	4 (4.8)	0.286	0.079–1.114	0.081
T	33 (39.3)	40 (23.5)			
C	51 (60.7)	130 (76.5)	0.476	0.271–0.835	**0.012**
IL-10 haplotype					
GCC GCC[d]	3 (7.1)	17 (20)	0.524	0.248–1.108	0.128
GCCACC, GCCATA[e]	16 (38.1)	35 (41.2)	1.138	0.533–2.427	0.848
ACC ACC, ACC ATA, ATA ATA[f]	23 (54.8)	33 (38.8)	0.286	0.079–1.114	0.081

The results that are statistically significant are typed in bold.
[a] $n = 42$.
[b] $n = 85$.
[c] Fisher's exact test.
[d] High gene expression.
[e] Low gene expression.
[f] Intermediate gene expression.
TGF-β1, transforming growth factor-β 1.

IFN-γ (+874) genotype

The frequencies of the genotypes of IFN-γ variant were TT (26.2%), TA (35.7%), and AA (38.1%) in the AML group. IFN-γ (+874) genotype and allele distribution showed no significant difference between the patients and the controls.

IL-10 genotypes (−1082, −819, −592) and haplotype

The frequencies of the AA, AG, and GG genotypes of IL-10 (−1082) were determined as 52.4.%, 40.5%, and 7.1%, respectively, in the AML group. IL-10 (−1082) AG variants showed no significant difference between the AML patients and the controls. However, frequency of the allele A was significantly higher in the AML group compared to controls (OR = 1.857, 95% Cl 1.051–3.278, $p = 0.038$). The genotypes CC, CT, and TT of IL-10 (−819) variant were 35.7%, 50.0%, and 14.3%, respectively. CC genotype and allele C were found significantly lower in patients with AML (OR = 2.450, 95% Cl 1.141–5.259, $p = 0.024$; OR = 0.476, 95% Cl 0.271–0.835, $p = 0.012$, respectively). CC, CT, and TT genotypes of IL-10 *(−592)* were found as 35.7%, 50.0%, and 14.3%, respectively. It was revealed that frequency of the CC genotype and allele C was significantly lower in the group of AML patients than in the control group (OR = 2.450, 95% Cl 1.141–5.259, $p = 0.024$; OR = 0.476, 95% Cl 0.271–0.835, $p = 0.012$, respectively). Although it is noteworthy that results of IL-10 (−819) and (−592) are identical, it has been shown that when we look at HaploTegV4, (−891) region resembles it in regard to promoter–histon relationship, but there are differences in terms of enhancer–histon, DDAse, protein binding, eQTL, and TF motifs.

Haplotype analysis for the *IL-10* gene (−1082, −819, −592) variants is shown in Table 2, and there was also no significant difference in the haplotype frequencies between the IL-10 and the control group.

TGF-β1 genotypes (codon 10 and 25) and haplotype

The frequencies of the genotypes in TGF-β1 (codon 10) were CC (28.6%), TC (57.1%), and TT (14.3%) in the AML group. No significant difference was found between the patients and the control group. The genotypes GG, GC, and CC of TGF-β1 (codon 25) variant were 85.6%, 7.2%, and 7.2%, respectively. TGF-β1 (codon 25) GC genotype was significantly lower in AML patients compared to the control group (OR = 4.000, 95% Cl 1.116–14.340, $p = 0.028$).

Haplotype analysis for the TGF-β1 gene (codon 10 and 25) variants is shown in Table 2. There was also no significant difference in the haplotype frequencies between the TGF-β1 and the control group.

Discussion

AML is an aggressive hematologic disorder, which is characterized by blockage in cell differentiation and accumulation of immature myeloid cells in the bone marrow (Chenjiao *et al.*, 2013; Mutlu *et al.*, 2014). In this study, we analyzed the association of AML susceptibility with polymorphic functional variants of TNF-α, IL-6, INF-γ, IL-10, and TGF-β1 genes. In addition, we evaluated haplotype analysis of IL-10 and TGF-β1 genes. Cytokines are small secreted proteins that regulate many cellular events, including hematopoiesis, immune responses, and inflammation. Dysregulation of cytokines and growth factor signaling are distinctive features in all types of leukemia and play a role in proliferation, survival, self-renewal, and resistance to chemotherapy. Many studies have demonstrated that cytokine gene variants cause interindividual variation of transcriptional regulation and subsequently alterations in serum levels of cytokines (Holleward and Bidwell, 2006; Winkler *et al.,* 2015).

TNF-α, encoding a proinflammatory cytokine secreted primarily by macrophages, is a critical factor in the pathogenesis of inflammatory autoimmune and malignant diseases (Feng *et al.*, 2014). The expression of adhesion molecules induced by TNF-α facilitates the invasion of metastatic tumor cells, and the serum TNF-α level increases in autoimmune diseases and many malignancies, including lymphomas (Zhai *et al.*, 2014). There is a promoter variant at nucleotide position −308 of the transcriptional start site of the gene and this is associated with autoimmunity and increased TNF-α production (Wihlborg *et al.,* 1999). It was shown that GA and AA genotypes lead to a higher rate of *TNF-α* gene transcription than wild-type GG genotype (Wilson *et al.*, 1997) does. It was reported that TNF-α 308 AA genotype and A allele were associated with gastric cancer (Lu *et al.,* 2010). Yang *et al.* reported that TNF-α-308 G/A variant was assumed to constitute a higher risk for hepatocellular carcinoma, especially in the Asian population (Yang *et al.*, 2011). In a study conducted with various ethnic groups, it was found that the AA genotype of TNF-α −308 may be a risk factor for breast cancer in African individuals (Shen *et al.*, 2011). Several studies have produced different results with regard to the prevalence of TNF-α −308 variant and its association with the risk of non-Hodgkin lymphoma (NHL) and ALL. Nasiri *et al.* found a statistically significant difference between ALL patients and the control group with respect to TNF-α −308 variant (Nasiri *et al.,* 2013). However, the studies of Zhao *et al.* in Chinese and Takeuchi *et al.* in German people showed no relationship between TNF-α −308 and ALL (Takeuchi *et al.*, 2002; Zhao *et al.*, 2003). Au *et al.* reported that TNF- α −308A was significantly associated with female CLL cases and had a strong negative prognostic impact on CLL in Chinese people (Au *et al.*, 2006). Stratified analyses suggested an increased risk of NHL with the presence of TNF-α −308 A allele among Africans and Caucasians, but a decreased risk among Asians (He *et al.*, 2014). TNF-α −308 variant was not found to be associated with other types of hematologic malignity, including CLL, B- CLL, hairy cell leukemia, and chronic myeloid leukemia (CML) (Demeter *et al.*, 1997; Bogunia-Kubik *et al.*, 2006; Lech-Maranda *et al.*, 2013; Pehlivan *et al.*, 2014). In our study, we have found that the frequency of TNF-α −308 GG genotype was significantly higher in AML patients than controls. The frequency of a low TNF-α producing GG genotype was significantly more common in the patients compared to the controls (Table 1).

IL-6 is a Th2 type cytokine with proinflammatory characteristics and affects a variety of inflammations, metabolic processes, and carcinogenesis. IL-6, produced by cells of the innate and adaptive immune system, leads to B-cell growth and differentiation (Sen *et al.*, 2011). Stimulating and maintaining the growth of AML blasts through the IL-6/IL-6 receptor signaling system are one of the effects of IL-6 on the growth of AML blasts. Serum levels of IL-6 constitute a significant factor for the prognosis of diffuse large cell lymphoma and chronic lymphocytic leukemia (Preti *et al.*,

1997; Fayad *et al.*, 2001). One of the most important variants in IL-6 is −174G to C substitution in the promoter region. This variant affects the transcription of IL-6 gene. GG and GC genotypes have been shown to have higher plasma levels of IL-6 while the presence of CC genotype leads to decreased expression of the IL-6 (Mandal *et al.*, 2014). IL-6 −174 variants were found to be associated with different types of cancers (Shi *et al.*, 2014; Bhat *et al.*, 2015; Liu *et al.*, 2015). Although it was detected that B-cell chronic lymphocytic leukemia (B-CLL) patients have more elevated plasma concentrations of IL-6 than healthy subjects, it was found that the allele frequencies of the IL-6 −174 variants were similar in patients and controls in same study (Hulkkonen *et al.*, 2000). Besides, there were some studies suggesting that IL-6 polymorphism was associated with hematologic malignity, including AML, CML, and multiple myeloma (Lehrnbecher *et al.*, 2005; Banu *et al.*, 2011; Pehlivan *et al.*, 2014). In contrast, there were also some studies showing that IL-6 −174 variant was not associated with multiple myeloma (Mazur *et al.*, 2005; Duch *et al.*, 2007; Aladzsity *et al.*, 2009). According to a study conducted in Turkey, although there were no statistically significant differences in the genotype or the allelic frequencies of the −174G/C variant between CLL, CML, AML cases and control groups, C allele of IL-6 had higher prevalence in CLL, CML, and AML patients (Mutlu *et al.*, 2014). Similarly, Ennas *et al.* reported that individuals homozygous for the IL-6 −174 C allele had an 11-fold increase in CLL risk (OR = 11.4, 95% CI 1.9, 69.4, $p = 0.008$) (Ennas *et al.,* 2008). Pehlivan *et al.* detected that IL-6 −174 GG genotype was significantly more frequent in CML patients than in controls ($p = 0.010$) (Pehlivan *et al.*, 2014). In our study, we found no difference in the genotype frequencies of IL-6 −174 between AML patients and healthy controls (Table 1).

IFN-γ, mainly produced by T and natural killer cells, plays a central role in the immune and inflammatory response. It also has antitumor and antiproliferative effects and is required for antitumor immunity (Vargas-Alarcor *et al.*, 2012). IFN-γ +874 polymorphism in the first intron is correlated with the level of IFN-γ production, where allele T is the high producer (Pravica *et al.*, 1999). Furthermore, there are some studies reporting that IFN-γ +874 polymorphism was associated with various cancer types, including cervical, breast, bladder, and hepatocellular cancers (Ahirwar *et al.*, 2009; Gangwar *et al.*, 2009; Li *et al.*, 2014; Zhou *et al.*, 2015), whereas there are studies showing that IFN-γ +874 variant is not associated with breast and lung cancer in certain populations (Gonullu *et al.*, 2007; Colakogullari *et al.*, 2008). Urbanowicz *et al.* reported that another polymorphism (+847 AT) in IFN-γ contributes to the pathogenesis of B-CLL (Urbanowicz *et al.,*2010). In a study, the frequencies of IFN-γ +874 TA genotype were significantly greater in the patients with CML (Basturk *et al.*, 2005), while it was found that, in another study, IFN-γ +874 genotypes and alleles were not significantly different in chronic phase CML patients in Turkey (Pehlivan *et al.*, 2014). Our results indicated that there is no significant association between the IFN-γ +874 variant and AML risk (Table 1).

IL-10 is a pleiotropic immune-regulatory cytokine, inhibiting the proinflammatory cytokine synthesis and the activation of Th1 cells and adhesion molecules (Vargas-Alarcon *et al.*, 2012). IL-10 is expressed predominantly in B lymphocytes. IL-10 is a cytokine that inhibits spontaneous AML blast proliferation and colony formation and decreases the secretion of IL-1α, IL-1β, TNF-α, granulocyte macrophage–colony-stimulating factor, depending on which exogenous growth factors are present (Bruserud *et al.*, 1995).Variants in the promoter region of IL-10 gene are likely to affect tumor development since they make changes on the levels of IL-10 in the serum or tumor microenvironment. There are previous studies, showing that there were several polymorphic sites in IL-10 gene promoter region in the transcription start site, including three balletic variants at positions −1082AG, −819CT, and −592AC. In various studies, it was reported that IL-10 variants are associated with various cancers such as lung cancer (Lan *et al.*, 2015), head and neck cancer (Niu *et al.*, 2015), esophageal cancer (Sun *et al.*, 2013), breast cancer (Wang *et al.*, 2014), and prostate cancer (Faupel-Badger *et al.*, 2008). Previous studies reported that IL-10 promoter variants were associated with high AML risk (Chenjiao *et al.*, 2013; Yao *et al.*, 2013; Fei *et al.*, 2015), CLL (Ovsepyan *et al.*, 2015), CML (Basturk *et al.*, 2005), and childhood ALL (Winkler *et al.*, 2015). In our case–control study, we genotyped our study population for three SNPs at positions −1082 A/G, −819 C/T, and −592 CA in the promoter region of IL-10 gene. The main finding of our study is that subjects carrying the −1082 A allele and −819 CC and −592 CC genotype showed a significantly decreased risk of AML. However, the haplotypes of IL-10 were not associated with AML (Table 2).

TGF-β signaling pathway is a significant antiproliferative and differentiation signal for hematopoietic progenitor cells as it is an effective factor for the prevention of progression through the cell cycle and promotion of differentiation (Dong and Blobe, 2006). The TGF-β superfamily has three main isoforms, namely TGF-β1, TGF-β2, and TGF-β3 (Gaur *et al.*, 2011). TGF-β1, a multifunctional cytokine, is secreted by several cell types, including peripheral blood mononuclear cells, endothelial cells, platelets, and plays a crucial role in the regulation of immunological homeostasis, angiogenesis, and cancer development (Mohy and Fouad, 2014). TGF-β1 is expressed in the most abundant form in endothelial cells, connective tissue, and hematopoietic cells (Gaur *et al.*, 2011). It was shown that exogenous TGF-β1 directly arrests growth and inhibits colony formation of $CD34^+$ human stem/progenitor cells *in vitro* (Dong and Blobe, 2006). Two coding variants, identified at exon 1 of TGF-β1 gene at positions 869 TC and 915 GC, cause amino acid substitutions in the signal peptide sequence at codon 10 (Leu → Pro) and codon 25 (Arg → Pro), respectively (Gaur *et al.*, 2011). It was found that both these polymorphic variants have a genetic control over the production capacity of TGF-β1 (Mazur *et al.*, 2006). Several studies conducted to identify the role of codon 10 TC variant of *TGF-β1* in cancer development and progression have produced inconsistent and insufficient results. Codon 10 allele C is associated with higher TGF-β1 mRNA and protein levels (Crilly *et al.*, 2002). It was reported that codon 10 allele C was correlated with increased susceptibility to nasopharyngeal (Wei *et al.,* 2007a) and esophageal squamous cell carcinoma (Wei *et al.*, 2007b) in the Chinese population. Furthermore, T allele at codon 10 was found to be associated with increased susceptibility to prostate cancer in the Japanese population (Li *et al.*, 2004), while no association was found between T allele at codon and non-Hodgkin lymphoma in the German population (Mazur *et al.*, 2006). It was determined that TGF-β1 codon 10 TT genotype was elevated in high-risk childhood ALL patients (Winkler *et al.*, 2015).

However, Pehlivan *et al.* reported that TT genotype in TGF-β1 codon 10 significantly decreased in patients with idiopathic thrombocytopenic purpura, compared to the control group (Pehlivan *et al.*, 2011). In the present study, the frequency of TGF-β1 codon 10 (+869 T/C) variant did not show significant difference between the groups. TGF-β1 codon 25 variant, located in the signal peptide sequence, alters peptide production, as this polymorphism affects a wide variety of cellular processes. At codon 25, the GG genotype produces significantly higher levels of TGF-β1 (Mazur *et al.*, 2006). Winkler *et al.* reported that patients with the GG genotype at TGF-β1 codon 25 had higher leukocyte counts in the initial diagnosis of ALL compared to patients with the GC and CC haplotypes (Winkler *et al.*, 2015). In the present study, a strong association was found between TGF-β1 codon 25 GC genotype and AML risk. In the AML group, the frequency of TGF-β1 codon 25 GC genotype was significantly lower than in healthy controls (OR:4.00; 95% CI:1.116–14.340; $p = 0.028$). TGF-β1 haplotype analysis did not show any significant difference between the patient and control groups.

Sample size is rather small in this study; however, the study of eight SNP in a single group offers significance. Relation of these SNPs with promoter histon, enhancer histon, DNAse, protein binding, eQTL, and Transcription factor motif in terms of HaploRegV4 program and bioinformatics has been reported in literature.

Conclusion

Despite that the subject of this study bears little novelty, it is the first trial in which polymorphisms were studied for the first time in Turkish AML patients. In conclusion, we have demonstrated that there is an association between certain cytokine gene functional variants and AML. These variants may be predictor for development of the AML. The incidence of AML has been gradually growing in Turkey, and larger studies are therefore necessary to investigate the role of variants involved in the pathogenesis of AML.

Author Disclosure Statement

No competing financial interests exist.

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