Association of TGFβ1 Gene (+915G>C) Polymorphism with Chronic Lymphocytic Leukemia

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ABSTRACT

TGFβ1 is an important cytokine acts as an antiinflammatory agent, and inhibits B cell proliferation. In patients with chronic lymphocytic leukemia (CLL), serum level of TGFβ1 are found elevated. Presence of G allele at position +915 in TGFβ1 gene results in arginine synthesis which is associated with higher expression of TGFβ1. We investigated the association of TGFβ1 +915G>C polymorphism with predisposition, clinical characteristics and laboratory findings of CLL. 50 CLL patients and 50 healthy controls were included in this study. Genotypes were determined by PCR-RFLP method. We couldn't find statistically significant differences between patient and control groups in terms of genotype distributions and allele frequencies. However, GC genotype frequency was slightly higher in CLL patients than healthy controls. Furthermore, TGFβ1 +915GC genotype was found associated with trisomy 12 (p= 0.007). Further studies are needed to clarify exact role of TGFβ1 +915G>C polymorphism in patients with CLL.

Keywords: Chronic lymphocytic leukemia, TGFβ1 polymorphism, Trisomy 12

ÖZET

TGFβ1 Geni (+915G>C) Polimorfizmi ile Kronik Lenfositik Lösemi Arasındaki İlişki

TGFβ1 antiinflamatuvar bir ajan rolüyle önemli bir sitokindir ve B hücre proliferasyonunu inhibe etmektedir. Kronik lenfositik lösemi (KLL) hastalarında ilginç olarak TGFβ1 serum seviyeleri artmış olarak saptanmıştır. TGFβ1 (+915G>C) polimorfizminde Arjinin aminoasidi ile ilişkili olan G alleli varlığında TGFβ1 ekspresyonu artmış olarak saptanmaktadır. Bu çalışmada TGFβ1 (+915G>C) polimorfizminin KLL hastalığına yatkınlık, klinik özellikler ve laboratuar bulguları ile ilişkisi incelenmiştir. Çalışmaya KLL tanısı almış 50 hasta birey ve sağlıklı kontrol grubu olarak 50 gönüllü birey dahil edilmiştir. TGFβ1 (+915G>C) genotipleri PCR-RFLP metodu ile belirlenmiştir. Çalışma sonucunda hasta grup ile sağlıklı kontrol grubu arasında genotip dağılımı ve allel frekansı açısından anlamlı bir fark saptanmamıştır. Bununla birlikte KLL hastalarında GC genotipi, istatistiksel olarak anlamlı olmasa da, kontrol grubuna göre daha fazla görülmüştür. Ayrıca TGFβ1 +915GC genotipi ile trizomi 12 varlığı arasında anlamlı bir ilişki saptanmıştır (p= 0.007). TGFβ1 +915G>C polimorfizmi ile trizomi 12 ve KLL arasındaki ilişkiyi açıklamak için başka çalışmalara da ihtiyaç vardır.

Anahtar Kelimeler: Kronik Lenfositer Lösemi, TGFβ1 polimorfizmi, Trizomi 12

INTRODUCTION

Chronic lymphocytic leukemia (CLL), neoplastic disease of CD5+ B lymphocytes, is the most common leukemia in western adult population. The incidence of CLL is 1.5-2.5/100.000, and the median age during diagnosis is 65 years.[1]

The etiology of CLL is still unclear. Any environmental factor, such as chemicals, drugs, and ionizing radiation, is not relevant to the disease.[2] CLL and other lymphoid malignencies, and also autoimmune diseases are found increased in relatives of CLL patients.[3-5] Furthermore anticipation phenomenon is reported in some familial cases.[6] These studies suggest that CLL has an important genetic basis.

In sipite of the strong findings of a genetic basis, no genes have been found consistenly associated with CLL. But polymorphism studies are rapidly increasing to determine candidate genes. Lymphotoxin alpha and Interferon gamma gene polymorphisms have been reported to be associated with CLL.[7-8]

TGFβ is a cytokine family which regulates many cellular responses such as apoptosis, cell growth, differentiation, and senescence.[9] TGFβ1 is the most important member of the TGFβ family. TGFβ1 polymorphisms were found associated with several malignencies.[10-13] TGFβ1 +915G>C polymorphism localised to the first exon leads to the Arginine(Arg)-Proline(Pro) substitution at codon 25. +915G allele involving Arg aminoacide is associated with higher expression of TGFβ1.[14]

In this study, we investigated the association of TGFβ1 +915G>C polymorphism with CLL. To the best of our knowledge, this is the first study investigated association between CLL and TGFβ1 polymorphisms.

PATIENTS AND METHODS

This study was approved by the Ethics Committee of the School of Medicine, Karadeniz Technical University, in accordance with the tenets of the Declaration of Helsinki. Fifty CLL patients were diagnosed at Karadeniz Technical University, Medical Faculty, Department of Hematology and 50 age- and sex-matched healthy subjects were enrolled who gave informed consent. CLL patients were characterized for age at diagnosis, gender, lymphocyte count, serum immunglobulin levels, and Rai stage. Diagnosis of CLL was established according to the National cancer Institute–Sponsored Working Group (NCI-WG) recommended criteria.[15] The clinical stage of the disease was determined according to the modified Rai classification.[16]

Genomic DNA was extracted from peripheral blood obtained from CLL patients and healthy volunteers using Fuji QuickGene-810 Nucleic Acid Isolation System. A 500 base pair fragment of the TGFβ1 which consists of +915G>C polymorphism was amplified using the following primer pair:[17] F:5' TTC AAG ACC ACC CAC CTT CT 3', R:5' TCG CGG GTG CTG TTG TAC A 3'.

PCR amplification of genomic DNA was carried out in a total volume of 25 μl containing 100 ng of genomic DNA, 200 μM of each dNTP, 1.0 μM of each primer, 2.0 mM MgCl2, 1 U Taq DNA polymerase, and 10X Taq buffer (Vivantis). The PCR protocol was as follows: initial activation step at 94^{0}C for 1 minute, followed by 35 cycles of denaturation at 94^{0}C for 30 seconds, annealing at 60^{0}C for 30 seconds, and extension at 72^{0}C for 30 seconds, and a final extension at 72^{0}C for 5 minutes. The PCR products were analyzed on 2% agarose gels.

PCR products were digested overnight at 37^{0}C for 1 hours with FseI (New England BioLabs [NEB], Ipswich, MA, USA) for TGFβ1 +915G>C genotypes. Digested products were seperated by electrophoresis on 3% agarose with ethidium bromide staining, and analysed by Gel Logic 212PRO imaging system (Carestream Health, Rochester, NY, USA). FseI digestion of PCR product yielded 500 bp for homozygote CC genotype, 318, and 182 bp for homozygote GG genotype, 500, 318, and 182 bp for heterozygote GC genotype (Figure 1). Genotyping results were confirmed with randomly selected PCR samples examined by DNA sequencing (Figure 2). Furthermore, genetic aberrations (13q14.3 deletion, 11q22.3 ATM deletion, 17p13.1

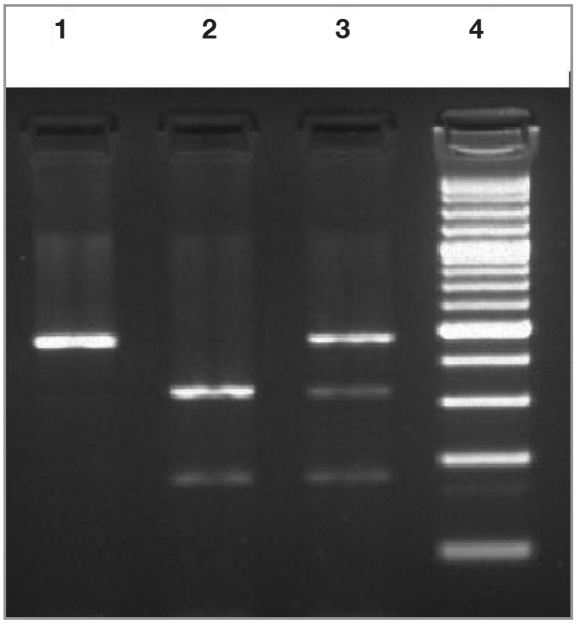

Figure 1. Agarose gel electrophoresis illustrating the TGFβ1 +915G>C genotypes

P53 deletion, trisomy 12, t(11;14)(q13;q32) IGH/CCND1 XT translocation) were analyzed by Fluorescence in situ hybridisation (FISH) techniques. For deletions, dual color locus specific probes (Vysis, USA); for translocation, dual color dual fusion locus specific probes (Vysis, USA); for trisomy, centromeric enumeration probes (Vysis, USA) were used. Lymphocyte culture was done from bone marrow or peripheral blood. FISH analysis was performed following the manufacturer's instructions.

Statistical Analysis

The software package SPSS for Windows 12.0 was used for statistical analysis. Chi-square test was used to calculate differences of the genotype distribution and the allele frequencies between patients with CLL and controls. Relationship between categorical variables and genotype distribution was analysed by the Chi-square test, while relationship between numerical variables and genotype distribution was analysed by the Mann Whitney U test. Because there was only one patient with CC genotype, this case was not included in comparison of genotypes. A two tailed $p< 0.05$ was considered significant.

RESULTS

Thirty five male, 15 female, a total of 50 CLL patients were included. The mean age at diagnosis

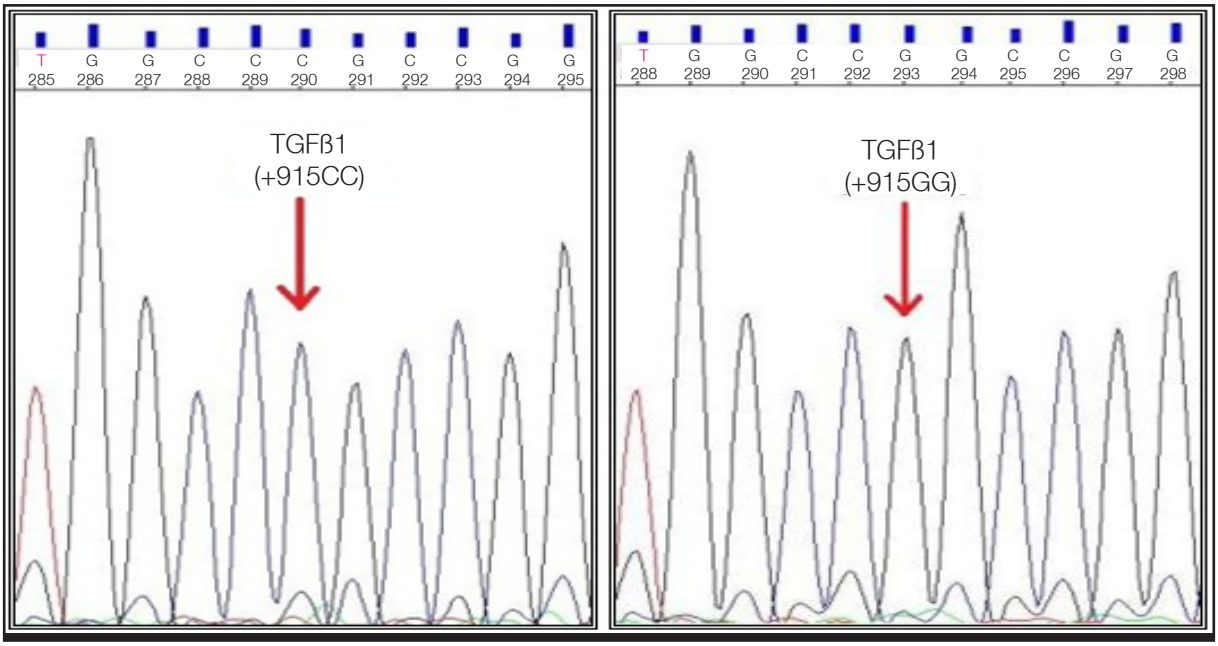

Figure 2. Sequence view of TGFβ1 +915CC and GG genotypes

Table 1. Comparison of patients with GG and GC genotypes

	GG (n= 36)	GC (n= 13)	p
Age	61	70	0.06
Gender (male/female)	27/9	7/6	0.29
Stage	2	1	0.82
Lymphocytes (/mm^3)	19.4	23.0	0.13
IgG	1070	1050	0.68
IgM	68.45	65.0	0.51
IgA	167	86	0.15
Trisomy 12 (%)	%9	%46	***0.007***
13q deletion	5	4	0.47
11q deletion	3	1	1.00

was 63 (range 32 to 84, SD= 11). The control group contained 35 male and 15 female healthy volunteers (mean age was 62, range 45 to 75, SD= 8). Characteristics of patients are summarised in Table 1.

The genotype distributions and allele frequencies of TGFβ1 +915G>C polymorphism among the patients and controls are shown in Table 2. No statistically significant differences were found between groups. But heterozygote GC genotype frequency was slightly higher in CLL patients than healthy controls. 13 patients (26%) showed GC genotype, tough, 10 healthy subjects (20%) had GC genotype.

We couldn't find any statistically significant relationship between clinical and laboratory findings (age at diagnosis, gender, Rai stage, lymphocyte count, serum immunglobulin levels) and genotypes, except for FISH findings.

Number of evaluated patients by FISH analysis for 13q deletion, 11q deletion, trisomy 12, 17p deletion, and 11;14 translocations were respectively 46, 45, 49, 44, and 45. 15 patients (33%) had 13q deletion, 6 patients (13%) had 11q deletion, 9 patients (18%) had trisomy 12, and 1 patient (2%) had 17p deletion. None of patients had 11;14 translocation. We did not find association of genotypes with 13q deletion, 11q deletion, 17p deletion, and 11;14 translocation, but detected a significant association between GC genotype and trisomy 12.

Patients with GC genotype had a higher frequency of trisomy 12. While 3 of 35 (9%) patients with GG genotype had trisomy 12, 6 of 13 (46%) patients with GC genotype had trisomy 12 (p= 0.007). There was only one patient with CC genotype, and this patient did not have trisomy 12. We evaluated association of trisomy 12 with age at di-

Table 2. Genotype distributions and allele frequencies of TGFβ1 +915G>C polymorphism in patients with CLL and controls

		Patients		Controls	
		n	%	n	%
Genotype	GG	36	72	40	80
	GC	13	26	10	20
	CC	1	2	0	0
Allele	G	85	85	90	90
	C	15	15	10	10

agnosis, gender, Rai stage, lymphocyte count and serum immunglobulin levels. No association was found. Logistic regression analysis revealed that trisomy 12 was associated with TGFβ1 +915G>C polymorphism independent of age and sex (Wald 128.7, 95%CI 11.6-16.5, p< 0.001).

DISCUSSION

To our knowledge, this is the first study which evaluated the association between TGFβ1 +915G>C polymorphism in patients with CLL. The results of our study does not suggest an association between TGFβ1 +915G>C polymorphism and CLL susceptibility. However, there was a non-significant tendency towards increased rate of TGFβ1 +915GC genotype in patients with CLL compared to controls. Furthermore, we detected a possible association between presence of trisomy 12 and TGFβ1 +915GC genotype, which might have importance in clinical practice.

TGFβ1 has an important role on permanence of immune homeostasis and suppression of autoimmunity. It inhibits proinflammatory cytokines, such as Tumor necrosis factor-alpha and IL-2. TGFβ1 suppresses Th1, Th2 cells, and APCs. And also inhibits B cell proliferation and production of antibody.[18-19] TGFβ1 +915G>C polymorphism localised to the first exon leads to the Arg-Pro substitution at the codon 25. +915G allele involving Arg aminoacide is associated with high expression level of TGFβ1.[14] High expression level of TGFβ1 is found commonly in patients with leukemia despite the negative role of TGFβ1 on hematopoiesis.[20] Babel et al. have found increased risk of post-transplant lymphoma in solid organ recipients with TGFβ1 +915CC genotype.[10] Mazur et al. reported that +915GG genotype was associated with two or more extranodal involvement in non-Hodgkin's lymphoma patients.[11] Patients with CLL presents with higher serum levels of TGFβ1 than healthy controls. In our study, TGFβ1 +915GG genotype which is associated with higher TGFβ1 levels tended to be lower in CLL patients compared to controls. This may infer a susceptibility of TGFβ1 +915 GC and CC genotypes to CLL.

Patients with early stages were reported to have higher serum levels of TGFβ1 than patients with advanced stages.[21-22] In vitro studies reported that TGFβ1 inhibits leukemic B cell proliferation in patients with CLL, but some patients with lack of TGFβ receptor type 1 expression showed resistance to the TGFβ.[23-24] In our study, CLL patients with GG genotype tended to be in higher stages compared to CLL patients with GC genotype. This finding is not consistent with the literature, because higher TGFβ1 levels are seen in earlier stages and GG genotype is reported to be associated with higher TGFβ1 levels.

In CLL patients, trisomy 12 frequency was found between 16% and 35%.[25-26] Dohner et al. reported that patients with trisomy 12 have longer overal survival compared to the normal karyotype but shorter than patients with deletion 13q.[27] On the contrary, Matutes et al. suggested that trisomy 12 is associated with advanced stage and poor prognosis.[28] In our study, there was no association between Rai stages and presence or absence of Trisomy 12.

Limitations of our study are the small sample size and cross-sectional nature of the study. Lack of assessment of expression levels of TGFβ1 is also a limitation of our study.

In conclusion, we could not find any association between TGFβ1 +915G>C genotypes and CLL susceptibility, but we detected an association between trisomy 12 and GC genotype. Further studies are needed to clarify the association of TGFβ1 +915G>C genotypes with trisomy 12 and investigate their exact role in patients with CLL.

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