CYTOKINE GENE POLYMORPHISMS IN TURKISH PATIENTS WITH CHRONIC MYELOID LEUKAEMIA AND IN HEALTHY CONTROLS

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ABSTRACT

Objective: *Cytokines that are released from activated lymphocytes, monocytes, and macrophages modify the intensity of immune inflammatory responses. Differences in cytokine production are due to sequence variants in cytokine genes. Cytokines are involved in all biological processes related to cell growth, cell differentiation, inflammation, and immunity. Early studies have demonstrated individual differences in cytokine production and an association between higher or lower cytokine production and disease. Chronic myelogenous leukaemia (CML) is a haematological malignancy that arises in haematopoietic stem cells with lymphoid and myeloid differentiation. Several studies have investigated the role of human leukocyte antigen (HLA) and cytokine gene polymorphisms in CML. The aim of this study was to analyse the role of cytokine polymorphisms in the aetiopathogenesis of CML. The susceptibility and protection of cytokine polymorphisms were evaluated.*

Material and Method: *A total of 300 adult healthy controls and 85 (70 adult and 15 paediatric) patients who were diagnosed with chronic-phase philadelphia chromosome (Ph)+ CML were included in this study. The typing was performed using the polymerase chain reaction-sequence specific primer (PCR-SSP) method. The allele and genotype frequencies of the following cytokine genes were determined: interleukin (IL)-6 (-174 G/C), IL-10 (-1082 G/A, -819 T/C, and -592 A/C), interferon-gamma (IFN-g) (+874 A/T), transforming growth factor-beta (TGF-β) (C/T codon 10, C/G codon 25), and tumor necrosis factor- alpha (TNF-α) (-308 G/A).*

Results: *We found that the frequencies of the TNF-α GA and IL-6 GC genotypes were higher in the healthy controls, and the differences between the CML patients and the controls were statistically significant. The analysis of the allele frequencies did not reveal statistically significant differences between the controls and the CML patients at 8 loci.*

Conclusion: *The TNF-α GA and IL-6 GC genotypes may be protective alleles in CML patients.*

Key Words: *Cytokine gene polymorphism, TNF-α, IL-6, chronic myeloid leukemia* ***Nobel Med 2014; 10(1): 74-78***

KRONİK MİYELOİD LÖSEMİLİ HASTALAR VE SAĞLIKLI KONTROLLERDE SİTOKİN GEN POLİMORFİZMİ

ÖZET

Amaç: Aktive edilmiş lenfositler, monositler ve makrofajlardan salınan sitokinler immün inflamatuar yanıtı düzenler. Sitokin üretimindeki farklılıklar sitokin gen dizi farklılıklarından kaynaklanmaktadır. Sitokinler hücre büyümesi, hücre farklılaşması, inflamasyon ve immünite ile ilgili tüm biyolojik süreçlerde yer alırlar. Daha önce yapılan çalışmalarda sitokin üretiminde bireysel farklılıklar ve hastalıklar ile yüksek veya düşük sitokin üretimi arasındaki ilişki gösterilmiştir. Kronik miyeloid lösemi (KML) hematopoetik kök hücrelerde lenfoid ve miyeloid farklılaşma ile ortaya çıkan hematolojik bir malignitedir. Çeşitli çalışmalarda KML de HLA ve sitokin gen polimorfizmlerinin rolü araştırılmıştır. Bu çalışmanın amacı kronik miyelositer lösemili hastalar ve sağlıklı bireylerde sitokin gen polimorfizmi etyopatogenezini incelemektir. Sitokin gen polimorfizmi yatkınlık/koruyuculuk yönünden değerlendirilmiştir.

Materyal ve Metod: Bu çalışmaya 300 yetişkin sağlıklı kontrol ve 85 (70 yetişkin ve 15 çocuk) kronik faz Ph + KML tanısı almış hasta dahil edildi. Sitokin gen polimorfizmi incelemek için kan örneklerinden DNA izole edildikten sonra [IL-6 (-174 G/C), IL-10 (-1082 G/A, -819 T/C, -592 A/C), IFN-g (+874 A/T), TGF-β (C/T codon 10, C/G codon 25) TNF-α (-308 G/A)] gen bölgeleri Polimeraz zincir reaksiyonu-allel spesifik primer (PCR-SSP) yöntemi kullanılarak tanımlandı.

Bulgular: Bu çalışmada TNF-α GA ve IL-6 GC genotiplerin sağlıklı kontrollerde sıklığının yüksek olduğu ve hastalar ile karşılaştırıldığında istatistiksel olarak anlamlı olduğu saptandı.

Sonuç: TNF-α GA ve IL-6 GC genotiplerin bu hastalığa karşı koruyucu olabileceği düşünülmektedir.

Anahtar Kelimeler: Sitokin gen polimorfizmi, TNF-α, IL-6, kronik miyeloid lösemi **Nobel Med 2014; 10(1): 74-78**

INTRODUCTION

Cytokines that are released from activated lymphocytes, monocytes, and macrophages modify immune inflammatory responses, bind to specific receptors in target cells, and function as chemical mediators between cells. Cytokines are involved in all biological processes related to cell growth, cell differentiation, inflammation, and immunity. Their role in these processes is diverse; specifically, cytokines may be pathogenic, protective, or, most often, innocent bystanders.[1] Recent studies have demonstrated that polymorphisms in the regulatory regions of cytokine genes affect gene transcription, which leads to variations in cytokine production.[2,3] Many of these polymorphisms occur in regulatory regions and result in high or low cytokine production. Allelic variants in the promoter regions of proinflammatory (tumor necrosis factor- alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin(IL)-6) and immunomodulatory (IL-10 and transforming growth factor-beta (TGF-β)) cytokine genes have been identified.[4,5] Cytokine polymorphisms are a major focus in the study of infectious diseases, such as listeria monocytogenes infection and mycobacterial infections, and chronic inflammatory diseases, such as chronic rheumatoid arthritis, Crohn's disease, and human cancers.[1,2,6]

High and low levels of cytokines and cytokine receptors, such as inappropriate levels of IL-2 and IL-2 receptors, are associated with the development of human cancers.[7] Other cytokines, including proto-oncogenes that encode receptor tyrosine kinases or their ligands, have been directly linked to the development of cancer.[8]

Chronic myelogenous leukaemia (CML) is a myeloproliferative disorder of clonal origin with an annual incidence of approximately 1.0-1.5 cases per 100,000 individuals in all countries where statistics are adequately reported.[9] CML is a haematological malignancy that arises in haematopoietic stem cells with lymphoid and myeloid differentiation potential.[10]

Many proteins may potentially act as leukaemia antigens in CML for major histocompatibility complex (MHC)-restricted cytotoxicity; however, the breakpoint cluster region-abelson murine leukemia viral oncogene homolog (bcr-abl) fusion protein has been the most extensively investigated. A significant amount of data suggests that bcr-abl junctional peptides can elicit both cluster of differentiation (CD) 4 and CD8 responses in normal healthy donors and in CML patients.[11] Several studies have investigated the role of human leukocyte antigen (HLA) and cytokine gene polymorphisms in CML.[12-14] However, studies have been unable to elucidate the role of cytokine genes due to major methodological problems, such as the typing approach, a single loci focus, and insufficient sample sizes of studied populations. The objective of this study was to analyse the role of →

cytokine polymorphisms in the aetiopathogenesis of CML in 85 Turkish patients with the disease.

MATERIAL and METHOD

Study Group

This study included 300 adult healthy controls and 85 (70 adult and 15 paediatric) patients who were diagnosed with chronic-phase Philadelphia chromosome (Ph)+ CML. In order to detect a meaningful difference of 0.25 between the two groups of alleles (Type 1 error=0.05, type 2 error=0.20, power=0.80, allocation ratio=0.28) 40 patients and 140 controls, is scheduled to be minimal sample size. Patient group is selected from the patients that CML diagnosis was approved by cytogenetics and/or by molecular genetics. The study was conducted in the Department of Haematology in the Medical School Hospital of Gaziantep University and at Our Children Leukaemia Foundation in Turkey. The study was approved by Gaziantep University's local institutional ethics committee (07-2007/40) and was performed according to the Decleration of Helsinki of 1975. Written informed consent was obtained from all the participants and from the parents of the paediatric patients. Of the 85 patients with CML, 32 were male and 53 were female. The age of these patients ranged from 10-78 years, with a mean age of 42.85 years (11±3.7). Instead of cytokine levels or concentrations in serum only cytokine gene polymorphisms were evaluated in this study so adult and the child group discrimination was not made as the gene polymorphisms are not related to age and sex.

Method

Mononuclear cells were obtained from Ethylene-diaminetetraacetic acid (EDTA)-treated peripheral venous blood, and genomic DNA was extracted using standardised procedures after DNA isolation. Cytokine genotyping was performed using the polymerase chain reaction sequence-specific primer method with the Cytokine Genotyping Tray (One Lambda, Inc., Canoga Park, CA, USA). The allele and genotype frequencies of the following cytokine genes were determined: IL-6 (-174 G/C), IL-10 (-1082 G/A, -819 T/C, and -592 A/C), IFN-γ (+874 A/T), TGF-β (C/T codon 10 and C/G codon 25), and TNF-α (-308 G/A).[15]

Statistical Analysis

The statistical analysis was performed using the SPSS® Statistical Package, version 14.0 (SPSS, Inc., Chicago, IL, USA), for Windows®. The alleles and genotypes in the patients and controls were compared using chi-square tests and two-tailed Fisher's exact tests. Fisher's exact tests were used if the observed value was less than five or the expected value was less than three. A p-value ≤0.05 was considered statistically significant. The data were analysed for goodness of fit based on Hardy-Weinberg Equilibrium (HWE).

RESULTS

The distribution of cytokine genotypes among the patients with CML and the healthy control group is summarised in Table 1. As the aim of this study was to evaluate the susceptibility and protection of cytokine polymorphisms and not directly related to the etiopathogenesis of the disease the cytogenetic and molecular results were not included. The cytokine genotype frequencies in the control group were consistent with HWE. The frequencies of the TNF-α GA (5.8% vs. 13.6%, *p*=0.05) and IL-6 GC (22% vs. 35%, *p*=0.02) polymorphisms were significantly lower in the CML patients than in the healthy controls. The frequency of IL-6 CC (11.7% vs. 5.6%) was higher in the CML patients than in the controls; however, this result was not statistically significant. The allele frequencies for the other cytokine gene polymorphisms did not vary significantly between the CML patients and the controls.

DISCUSSION

In addition to histocompatibility disparity, other genetic factors may predispose patients to developing CML, such as T helper type 1 and 2 (Th1 and Th2) cytokines and their gene polymorphisms. The expression and secretion of cytokines depend on genetic polymorphisms (nucleotide variations) within the promoter region of cytokine genes or other regulatory sequences. The majority of the polymorphisms that have been described to date are single nucleotide polymorphisms (SNPs).[16]

TNF is a pleiotropic cytokine that activates various immunological and inflammatory host defence responses and is considered an important mediator of protection from parasitic, bacterial, and viral infections. TNF-α, as a crucial pro-inflammatory cytokine which was firstly isolated as an anticancer cytokine, plays several important roles in the establishment of inflammation, cell growth control, induction of pathogenic stimuli, and as a major therapeutic role.[17-19] TNF plays a role in a wide variety of diseases, including rheumatoid arthritis and Hodgkin's disease.[2] The G to A polymorphism at position 308 of the TNF-α promoter is associated with increased TNF-α production and allograft rejection in renal, heart, and lung transplants.[14,20] →

In this study, we analysed allele frequencies in the cytokine gene region and did not detect any significant differences between the patients and the healthy controls. The genotype analysis indicated that the frequencies of the TNF-α GA and IL-6 GC genotypes were higher in the healthy controls, and the differences between the CML patients and the controls were statistically significant.

Olomolaiye et al. show that elevated IL-6 levels are associated with haematological malignancies, especially multiple myeloma. They detected a G/C polymorphism and a base difference in the promoter region of IL-6, which was positioned at -174. Researchers have reported that the C allele at this locus was correlated with low plasma IL-6 levels.[21] Functional studies have demonstrated that polymorphisms at position -174 changed the expression ratio of the IL-6 gene. Rein et al. found that individuals with a C allele at -174 had lower IL-6 expression than individuals with a G allele at this locus. In addition, they found that a G/C base variation at position -174 resulted in a binding site for the NF-1 transcription factor.[21-23] In this study, we concluded that there were no significant differences between the percentages of patients and controls with the C allele in the IL-6 gene region. However, the frequency of GC genotypes in the controls was higher than that in the CML patients. Amirzargar et al. studied 30 CML patients in Iran and found that the production of TGF-β in CML patients was higher and the production of IL-4 and IL-10 was lower compared with normal subjects.[14] Singer et al. studied cytokine serum levels but not the gene polymorphisms in 25 CML patients, 10 controls and found that there is a correlation between IL-6 and IL-18 supposing that there is a synergistic role of these cytokines in disease progression in chronic phase.[24]

In a study of 30 CML patients in Turkey, IL-10 GCC/ATA and IFN-γ TA were risk factors for the disease, whereas IFN-γ AA was a protective factor.[13] The relationship between the TGF-β and IL-10 genotypes, which was found as a risk or protective factors in previous studies by Amirzargar et al. and Basturk et al., were not detected significant in this study.[13,14] However, we find an association between several cytokine gene polymorphisms and CML. Therefore, the TNF-α GA and IL-6 GC genotypes may be valuable protective determinants of the development of CML. Further research is needed to understand the aetiopathogeny of CML.

Table 1: The distribution of cytokine genotypes among the chronic myelogenous leukaemia (CML) patients and the control group

GENOTYPE	CML		CONTROL							
	n	%	n	%	OR	95% CI		p	Control	
						Down	Up		HW-x^2	P
TNF-α										
AA	1	1.18	4	1.33	0.88	0.09	7.99	1.00	2.36	p>0.05
GA	5	5.88	41	13.67	0.39	0.15	1.03	0.05		
GG	79	92.94	255	85.00	2.32	0.95	5.65	0.07		
IL-6										
CC	10	11.76	17	5.67	2.22	0.97	5.04	0.08	0.38	p>0.05
GC	19	22.35	106	35.33	0.52	0.30	0.92	0.02		
GG	56	65.88	177	59.00	1.34	0.81	2.22	0.26		
IFN-g										
AA	23	27.06	104	34.67	0.69	0.49	1.19	0.23	0.06	p>0.05
TA	47	55.29	138	46.00	1.45	0.89	2.35	0.14		
TT	15	17.65	58	19.33	0.89	0.47	1.67	0.87		
TGF-β 10										
CC	16	18.82	55	18.33	1.03	0.55	1.91	1.00	0.67	p>0.05
TC	47	55.29	155	51.67	1.15	0.71	1.87	0.62		
TT	22	25.88	90	30.00	0.81	0.47	1.40	0.50		
TGF-β 25										
CC	1	1.18	4	1.33	0.88	0.09	7.99	1	0.41	p>0.05
GC	10	11.76	52	17.33	0.63	0.30	1.31	0.24		
GG	74	87.06	244	81.33	1.54	0.76	3.10	0.25		
IL-10 -1082										
AA	41	48.24	122	40.67	1.36	0.83	2.20	0.21	1.4	p>0.05
GA	33	38.82	131	43.67	0.81	0.50	1.34	0.45		
GG	11	12.94	47	15.67	0.80	0.39	1.62	0.6		
IL-10 -819										
CC	40	47.06	153	51.00	0.85	0.52	1.38	0.54	0.52	p>0.05
CT	40	47.06	126	42.00	1.22	0.75	1.99	0.45		
TT	5	5.88	21	7.00	0.83	0.30	2.27	1		
IL-10 -592										
AA	5	5.88	21	7	0.83	0.30	2.27	1	0.52	p>0.05
CA	40	47.06	126	42	1.22	0.75	1.99	0.45		
CC	40	47.06	153	51	0.85	0.52	1.38	0.54		

TNF-α:Tumor necrosis factor-alpha, IL-6:Interleukin-6, IFN-g:Interferon-gamma, TGF-β:Transforming growth factor-beta, IL-10:Interleukin-10, OR:Odds ratio, HWx^2: Hardy-Weinberg Equilibrium chi-square

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