RESEARCH ARTICLE

Editorial Process: Submission:07/19/2023 Acceptance:02/11/2024

Interleukin-6 And Interleukin-10 Polymorphisms In Chronic Lymphoid Leukemia Patients

Sanem Yildiz, Sibel Bayil Oguzkan*, Mehmet Ozaslan, Alperen Kizikli, Ibrahim Halil Kilic, Mehmet Yilmaz

Abstract

Objective: A major part of the cytokines secreted from the immune system are interleukins (IL) and their main role is to stimulate the immune system cells. Therefore the genotypic effects of *IL-6* and *IL-10* on the immune system in CLL were investigated in the study. **Method:** For this purpose 100 patients diagnosed with CLL and 70 healthy individuals with no cancer history were included in the study. Polymorphisms at *IL10* and IL 6 promoter regions (1082 A\G and 819 C\T) and *IL6* (174 G\C) polymorphisms were analyzed with RT-PCR. Genotype and allele frequencies were directly calculated. **Result:** In 100 CLL patients, 45 wild type AA, 40 AG and 15 mutant type GG genotypes were found for the *IL 10* 1082 A\G region. Genotypic distribution of *IL10* 819 C\T region was determined as CC, BT and TT genotypes in 37, 50 and 13 patients, respectively. In IL 6 174 G\C region, GG, GC and CC genotypes were determined in 62, 30 and 8 patients, respectively. There is no statistically significant difference between the CLL patients and control groups in terms of *IL10* 1082 A\G, 819 C\T and IL 6 174 G/C regions ($p > 0.05$). As a result of the allele frequency calculation of the *IL 10* 1082 region, the values obtained were A (0.65), G (0.35) for the patient group and (0.61) and G (0.31) for the control group. 819 region allele frequencies were C (0.57) and T (0.33) in the patient group and C (0.48) and T (0.32) in the control group. The *IL6* 174 region was calculated as G (0.82), C (0.28) in the patient group and G (0.63), C (0.23) in the control group. Given the number of patients within the limits of this study, *IL 10* and *IL 6* genotype frequencies do not seem to be statistically related to CLL patients. **Conclusion:** Mutant alleles of all interleukin SNPs were determined at a higher frequency in the patient group as compared to the control group. Therefore, a potential correlation between the SNPs of these interleukins and CLL can be determined in future studies with a higher number of samples.

Keywords: *IL10*- *IL6*- CLL- RT-PCR

Asian Pac J Cancer Prev, **25 (2)**, 461-464

Introduction

Leukemia is a cancer type that manifests itself with the abnormal proliferation of blood cells, especially leucocytes, and affects the blood generation system in the body. Leukemias are classified based on the spread and development characteristics of the tumor as acute or chronic. In chronic lymphocytic leukemia (CLL), cancer cells are found in bone marrow, blood and lymph nodes [1]. The most common one is the chronic lymphocytic leukemia with an incidence rate of 30% among individuals [2]. Chronic lymphocytic leukemia (CLL) is a leukemia type that is characterized by an abnormal increase in mature-looking small B lymphocytes in the peripheral blood, bone marrow or lymphoid tissue. In addition, autoimmune disorders caused by a deterioration in the immune system are commonly seen in CLL patients [3]. Single nucleotide polymorphisms (SNPs), which involve changes in the DNA sequence seen in more than 1% of the general population, except for mutations in the human genome, play an important role in the formation of cancer [4]. We can define SNPs as the changes that show the nucleotide differences between the alleles in the same region in the DNA of two individuals. Up until today, many SNPs have been associated with cancer. We can say that SNPs are an important factor in the formation of cancer. SNPs can also affect clinical states such as the prognosis of disease, success of treatment, drug resistance or resistance to a viral infection [5].

Cell proliferation, differentiation, apoptosis mechanisms and polymorphisms that occur in the structure of cytokines and that play a role in regulation of the immune system are other important genetic factors in the emergence of CLL [6]. An important part of the cytokines that are secreted from the immune system consists of interleukins. According to 2018 data, cancer is one of

Department of Medical Services and Techniques, Vocational School of Health Services, University of Gaziantep, Gaziantep, Turkey.
**For Correspondence: bayil@gantep.edu.tr*

the main causes of death globally. It accounts for 18.1 million new cases and an estimated 9.6 million deaths. People with leukemia make up 437,033 out of 11 million of the new cases, and 309,006 of the estimated mortality count [7]. Even though male gender, advanced age, and a cancer history in the family increase the risk of cancer, the etiology of the CLL disease is not exactly known [8]. Chromosome mutation studies have shown the correlation of CLL with 13q14 and 11q22-q23 deletion, trisomy 12, 17p13 deletion [6, 9]. Ovsepyan et al. studied *IL 10* promotor region gene polymorphism in CLL patients. It was found that allele 1082A and its genotypes (1082AA / 1082AG) are associated with a risk of chronic lymphoid leukemia [10]. Having found that homozygote *IL6*-174C allele poses a risk in the development of CLL in their study performed to examine the interleukin-6 (*IL6*) gene polymorphism, Ennas et al. observed that other SNPs had no correlation with CLL risk [11].

Zhao et al. evaluated the prognosis of 174G/C, which is a single nucleotide polymorphism (SNP) in *IL-6*, on non-small cell lung cancer (NSCLC) patients. GG or GC genotypes were determined at a high rate whereas CC genotype was found at a low rate. The study showed that patients with G allele (CG / GG) had a shorter survival time than patients with CC genotype [12]. Studies conducted show that research undertaken to understand polymorphisms and CLL development are insufficient, therefore we evaluated the *IL10* 819 C/T, *IL10* 1082 A/G region and *IL6* 174 G/C cytokines that are associated with both CLL and other cancer types in patients diagnosed with CLL in this study.

Materials and Methods

Study population

In order to analyze the *IL10* 819 C/T, *IL 10* 1082 A/G and *IL 6* 174 G/C promotor region gene polymorphisms in Chronic Lymphoid Leukemia (CLL) patients, approval was given by the Clinical Trials Ethics Committee of Gaziantep University in decision no 321/2016. 100 individuals over the age of 18, who had been admitted to Gaziantep University Sahinbey Research and Application Hospital Hematology Department had been diagnosed with Chronic Lymphoid Leukemia were included in the patient group and 70 healthy individuals over the age of 18, who were residing in Gaziantep and who had no history of cancer were included as the control group in our study. Blood samples included in the study were collected from the Gaziantep University Sahinbey Research and Application Hospital between 2016-2017.

Blood samples

Blood samples obtained were kept at -20 °C until the date of the study in order to investigate *IL10* 819 C/T, *IL 10* 1082 A/G and *IL 6* 174 G/C promotor region gene polymorphisms. When the study commenced, manual DNA isolation was performed on the blood samples from the control group and the patient group. Genomic DNA was isolated using the salting-out method from whole blood. Quantitative and purity analyses of the DNA samples were performed by obtaining readings of the diluted samples at 260 and 280 nm wavelengths in a UV spectrophotometer. Absorption values (A260) measured at 260 nm wavelength and absorption values (A280) measured at 280 nm wavelength showed the amount of DNA and amount of protein in the DNA solution, respectively. The purity grade of the DNA sample was determined by calculating the ratio (A260 / A280) between the values read at 260 and 280 nm wavelengths [13].

Genotyping

The real time online PCR method was used in the Real-time PCR device for the DNA samples of the patient and control groups selected for our study. *IL 10* 1082 A\G, IL 10 819 C\T and *IL 6* 174 G\C genotype distribution were studied with SNP Identification Kit. Genotype and allele frequencies were calculated directly.

Statistical analysis

Data are presented as the mean± SD or percentage. Statistical analysis was performed using SPSS for Windows version 16. Chi-square test or fisher'sexact test were used for calculationof the significanceondifferences in genotype and allelefrequencies. A value of $p<0,05$ consideredstatistically significant and for all stattisticaltests were two-sided.

Results

100 individuals (34 females, 66 males) who had applied to Gaziantep University Şahinbey Research and Application Hospital Hematology Department, were diagnosed with Chronic Lymphoid Leukemia and who had blood tissue samples registered in the archive were included in the patient group and 70 healthy individuals (24 females, 46 males) who had no cancer history were included in the control group in our study. The demographic data (gender and age) about the patient and control groups is shown in Table 1. Genotype distributions of *IL 10* and *IL6* determined with RT-PCR are as shown in Table 2. When the genotypicdistribution of the patient group is examinedIL 10 1082 A\G region, 45 wild-type AA (%45), 40 AG (%40) and 15mutant-type GG (%15) genotypes, 819 C\T regions of *IL10* was CC in 37(%37) patients, CT in 50 (%50) patients and TT genotype in 13(%13) patients was found. *IL 6* 174 G\C region was detected in GG in 62 (%62) patients, GC in 30 (%30) patients and CC genotypes in 8 (%8) patients. There was no statistically significant difference between CLL patients and controlgroups of *IL10* 1082 A\G and 819 C\T and IL 6 174 G/C regions ($p> 0.05$).As a result of calculation of allelefrequency of IL 10 1082 region, for the patient group

Table 1. PatientandControlGroupDemographicdistrubition

Patients and Control Group	Gender	n	Percentage (%)	Age (Mean (±) SD)
Patients group	Woman	34	34%	64,01±10,81618694
	Man	66	66%	
Control group	Man	24	34.28%	45,24±11,61692297
	Woman	46	65.71%	

n, sample number; SD, Standart Deviation

Table 2. Genotypedistributions and Allele Frequencies of *IL10* 819 C/T, *IL 10* 1082 A/G ve *IL 6* 174 G/Cpolymorphisms in Patients and Controls Group

P Patients	(n = 100)	Control (n = 70)	p
IL-6 -174 Genotypes(rs1800795)			
GG	62 (%62)	43 (%61.4)	
GC	30 (%30)	24 (%34.2)	0.537
CC	8 (%8)	3 (%4.2)	
IL-6 -174 Alleles			
G	0.82	0.63	
C	0.28	0.23	
IL-10 -819 Genotypes(rs1800871)			
CC	37 (%37)	28 (%40)	
CT	50 (%50)	30 (%42.8)0.636	
TT	13 (%13)	12 (%17.1)	
IL-10 -819 Alleles			
C	0.57	0.48	
T	0.33	0.32	
IL-10-1082 Genotypes(rs1800896)			
AA	45 (%45)	41 (%58.5)	
AG	40 (%40)	18 (%25.7)	0.122
GG	15 (%15)	11 (%15.7)	
IL-10-1082 Alleles			
A	0.65	0.61	
G	0.35	0.31	

A (0,65), G (0,35), control group (0,61) and G (0,31) were obtained. The frequencies of C (0,57) and T (0,33) were found in the patient group of 819 region as C (0,48) and T (0,32) in the control group. *IL6* 174 region was calculated as G (0,82) C (0,28) in the patient group and G (0,63), C (0,23) in the control group.

Discussion

Cytokines play a role in regulation of the immune system, apoptosis, proliferation, inflammatory reactions and differentiation of lymphoid cells. Interleukin 10 is a cytokine that plays a key role in the immune system. The polymorphisms known as SNPs that occur in the structure of cytokines are reported in various sources to be effective in the clinical development and prognosis of CLL [14,15,16].

In a study they conducted, Mutlu et al. investigated the correlation between IL 6 174G/C gene polymorphism and chronic lymphocytic, chronic myeloid and acute myeloid leukemia in the Turkish population. 23 CLL, 25 CML and 17 AML patients and 30 healthy individuals were included in the study. Single nucleotide polymorphisms (SNPs) were genotyped with the PCR-RFLP method. The C allele frequency was found to be high. These results show that the C allele is associated with CLL, CML and AML risk in Turkish patients. However, no statistically significant difference was found between the CLL, CML and AML cases in terms of genotype of the -174G/C polymorphism or allele frequencies. As such, no statistically significant difference was seen in the IL 174 G/C polymorphism in our study [17].

Fei et al. investigated the correlation between IL 10 (IL-10) -1082G/A (rs1800896), -819T/C (rs1800871) and -592A/C (rs1800872) polymorphism and acute myeloid leukemia (AML) risk in the Chinese population. The PCR-RFLP technique was used in the study that included 167 patient group and 328 control group individuals. The study showed that individuals with rs1800871 CC genotype and C allele carried a substantial risk of AML. We identified the potential correlation in the same regions with CLL in this study [18].

Al Suhaibani et al. examined the polymorphisms at the *IL-6* (-174G/C) and *IL-10* (-1082G/A) promotor regions in their study which included 80 women who were pathologically diagnosed with breast cancer and 80 healthy women. For the *IL-6* -174G/C polymorphism, CC genotype was found to a higher degree in the patients compared to the control group. No difference was found in the genotype distribution between the patients and control group individuals for the *IL-10* -1082G/A polymorphism. Additionally, it was seen that the tumor size was larger in patients with AA genotype compared to patients with the AG or GG genotype. There is no significant difference between the control and patient groups for the same regions in this study which included CLL patients [19].

In prostate cancer patients whose *IL-6* (-174G/C) region was studied by another investigator, no significant correlation was found between the *IL-6* 174 G/C polymorphism and the risk of prostate cancer. In our study, there was no statistical correlation between genotype frequencies and CLL in the *IL 6* 174 G/C polymorphism studied for CLL patients [20]. In their study on whether the interleukin-10 (*IL-10*) polymorphisms created a risk of craniocervical cancer (HNC), Yu-Ming Niu et al. found a significant correlation between *IL-10*-1082A/G polymorphism and HNC risk, contrary to our study [21].

In conclusion, no statistically significant correlation was found between the SNPs in two regions of *IL 10* and a single region of *IL6*, and CLL in our study. Due to the limited number of patients in the study, this study can be evaluated as a basis for further studies by increasing the number of patients diagnosed with CLL and by examining different cytokine polymorphisms.

Author Contribution Statement

None.

Acknowledgements

This article IX. It was presented orally at the International Eurasian Hematology Oncology Congress.

Funding

This study was carried out by Gaziantep University Scientific Research Project Unit. Supplemented with reference number FEF. YLT.17.18.

Competing interest

The authors are not aware of any other potential conflict of interest associated with this manuscript.

Data Availability

All relevant data are within the paper and its supporting information files

Ethical committee that approved the research

Gaziantep University clinical research ethics committee

References

1. Montserrat E, Moreno C. Chronic lymphocytic leukaemia: A short overview. Ann Oncol. 2008;19 (Suppl 7):320-5. https://doi.org/10.1093/annonc/mdn460.
2. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med. 1995;333(16):1052-7. https://doi.org/10.1056/nejm199510193331606.
3. Dameshek w. Chronic lymphocytic leukemia—an accumulative disease of immunologically incompetent lymphocytes. Blood. 1967;29(4):566-584. Blood. 2016;128(4):465-. https://doi.org/10.1182/blood-2016-05-716159.
4. Ekmekçi A, Konaç E, Önen IH. Gene polymorphısm and genetic susceptıbılıty to cancer. Marmara Med J. 2008;21(3):282-95.
5. Wang Z, Moult J. Snps, protein structure, and disease. Hum Mutat. 2001;17(4):263-70. https://doi.org/10.1002/humu.22.
6. Caporaso N, Goldin L, Plass C, Calin G, Marti G, Bauer S, et al. Chronic lymphocytic leukaemia genetics overview. Br J Haematol. 2007;139(5):630-4. https://doi.org/10.1111/j.1365-2141.2007.06846.x.
7. L. Siegel MPH, Kimberly D. Miller MPH, Ahmedin Jemal DVM. Cancer Statistics, 2018. American Cancer Society, 68: 1, 2018. doi: 10.3322/caac.21442. Epub 2018 Jan 4.
8. Ries LAG, Harkins D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, et al. Seer cancer statistics review, 1975–2003. National cancer institute, bethesda, md. 2006.
9. Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910-6. https://doi.org/10.1056/nejm200012283432602.
10. Ovsepyan VA, Gabdulkhakova A, Shubenkiva AA, Zotina EN. Role of interleukin-10 gene promoter region polymorphism in the development of chronic lymphoid leukemia. Bull Exp Biol Med. 2015;160(2):275-7. https://doi.org/10.1007/s10517-015-3148-6.
11. Ennas MG, Moore PS, Zucca M, Angelucci E, Cabras MG, Melis M, et al. Interleukin-1b (il1b) and interleukin-6 (il6) gene polymorphisms are associated with risk of chronic lymphocytic leukaemia. Hematol Oncol. 2008;26(2):98-103. https://doi.org/10.1002/hon.843.
12. Zhao K, Xu J, Tian H. Correlation analysis between an il-6 genetic polymorphism and non-small cell lung cancer prognosis. Genet Mol Res. 2016;15(1):15017021. https://doi.org/10.4238/gmr.15017021.
13. Carpi FM, Di Pietro F, Vincenzetti S, Mignini F, Napolioni V. Human DNA extraction methods: Patents and applications. Recent Pat DNA Gene Seq. 2011;5(1):1-7. https://doi.org/10.2174/187221511794839264.
14. Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: Correlation with phenotypic characteristics and outcome. Blood. 2001;97(1):256-63. https://doi.org/10.1182/blood.v97.1.256.
15. Lech-Maranda E, Mlynarski W, Grzybowska-Izydorczyk O, Borowiec M, Pastorczak A, Cebula-Obrzut B, et al. Polymorphisms of tnf and il-10 genes and clinical outcome of patients with chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2013;52(3):287-96. https://doi.org/10.1002/gcc.22028.
16. Caimi PF, de Lima M. Genetic polymorphisms, chronic lymphocytic leukemia, and the future: Are we there yet? Rev Bras Hematol Hemoter. 2014;36(1):5-6. https://doi.org/10.5581/1516-8484.20140003.
17. Mutlu P, Yalcin S, Elci P, Yildirim M, Cetin AT, Avcu F. Association of -174g/c interleukin/6 gene polymorphism with the risk of chronic lymphocytic, chronic myelogenous and acute myelogenous leukemias in turkish patients. J buon. 2014;19(3):787-91.
18. Fei C, Yao XM, Sun Y, Gu XZ, Yu LQ, Lai X. Interleukin-10 polymorphisms associated with susceptibility to acute myeloid leukemia. Genet Mol Res. 2015;14(1):925-30. https://doi.org/10.4238/2015.February.2.15.
19. AlSuhaibani ES, Kizilbash NA, Malik S, Dasti JI, Al Beladi F, El-Morshedi N. Polymorphisms in promoter regions of il-6 and il-10 genes in breast cancer: A case-control study. Genet Mol Res. 2016;15(1). https://doi.org/10.4238/gmr.15017360.
20. Magalhães JF, Cortinhas AJ, Albuquerque CM, Baptista CS, Ribeiro R, Viegas C, et al. Interleukin-6 gene -174g>c and -636g>c promoter polymorphisms and prostate cancer risk. Mol Biol Rep. 2013;40(1):449-55. https://doi.org/10.1007/s11033-012-2079-9.
21. Niu YM, Du XY, Cai HX, Zhang C, Yuan RX, Zeng XT, et al. Increased risks between interleukin-10 gene polymorphisms and haplotype and head and neck cancer: A meta-analysis. Sci Rep. 2015;5:17149. https://doi.org/10.1038/srep17149.