Abstract. Aim: The aim of the present study was to investigate the association between Paraoxonase 1 (PON1) gene polymorphisms Q192R, and L55M in patients with Chronic Myelogenous Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL) patients. Materials and Methods: We analyzed samples from 60 patients with CML, 60 with CLL and 84 healthy controls. Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP) was performed and samples were run in agarose gel. Results: We found statistically significant results showing an increase in both the RR genotype (p=0.044) and the R allele (p=0.011) for PON1 Q192R, and an increase in the MM genotype (p=0.007) and a decrease in the LL genotype (p=0.004) and R allele (p=0.001) in PON1 L55M in patients with CLL. Conclusion: We concluded that both the Q192R gene polymorphism with an increase in the genotype R allele, and the M/L55 with an increase in the MM genotype play a role in CLL susceptibility, and a decrease in the LL genotype can act against disease in the Turkish population.

Reactive oxygen species (ROS) can oxidize biological molecules that mediate carcinogenesis by causing both metabolic malfunction, and damage to DNA. Human serum paraoxonases (PONs) play a role in anti-oxidant defense and protect cells against ROS (1). PON1 is a serum esterase synthesized in the liver. PON1 is a member of a family of proteins that include PON2 and PON3, the genes for which are clustered on the long arm of the human chromosome 7(q21.22) (2). PON1 has two common polymorphisms that influence both PON1 concentration and activity. Epidemiological and molecular studies have shown that there are two important genetic polymorphisms in positions 55 and 192 of the PON1 gene. These two polymorphisms arise from the substitution of amino acids in positions 55 and 192. The substitution of glutamine (Q genotype) in position 192 of the PON1 gene by the arginine (R genotype) leads to the first polymorphism rs662 (Q192R). Similarly, the substitution of leucine (L genotype) in position 55 by methionine (M genotype) leads to the second polymorphism rs854560 (L55M). The most common genotype is homozygous QQ, the second is heterozygous QR, and the least common is homozygous RR. The hydrolyzing activity of the protein encoded by R is 8-fold more than that encoded by the Q allele. This polymorphism also affects the serum protein concentration. Homozygous RR individuals have higher enzyme concentrations than the homozygous QQ individuals (3, 4). In addition to the polymorphisms at these positions, five polymorphisms were reported for the PON1 promoter region. Due to these genetic polymorphisms, PON1 activity may differ by up to 10- to 40-fold among different individuals and populations (5, 6).

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm related to the presence of the Breakpoint Cluster Region (BCR) – Abelson (Abl)1 fusion gene, which is linked to t(9;22)(q34;q11). It originates from an abnormal hematopoietic stem cell that is characterized from its normal counterpart by long-term self-renewal and multi-lineage differentiation. Both leukemia and quiescent normal hematopoietic stem cells preferentially reside in the osteoblastic niche. Mesenchymal stromal cells are located near them, playing a critical role in their regulation (7).
B-Cell chronic lymphocytic leukemia (CLL) is the most common type of human leukemia (8). This disease is characterized by the monoclonal expansion of the B-lymphocytes in the peripheral blood, bone marrow, and lymphoid organs, and by an indolent course, which ultimately becomes aggressive and invariably lethal. CLL cells have a low proliferative rate and a prolonged life-span, suggesting that their primary alteration may be a defect in apoptosis (9). The cellular origin of CLL is unknown, and has been the subject of recent controversy. CLL cells are characterized by the expression of the cell surface markers CD5, CD23, CD19, and low levels of IgM/IgD, a pattern not shared by any known B-cell subpopulations (9-12). CLL is not associated with reciprocal balanced chromosomal translocations, but rather with specific deletions (13), suggesting the loss of known tumor-suppressor genes. The 13q14 deletions are the most common chromosomal alterations in CLL (8). Palamarchuk et al. reported that miR-15/16 is a target of 13q14 deletions, and plays a tumor-suppressor role by targeting B-cell lymphoma 2 (BCL2). In addition, they found that deleted in lymphocytic leukemia, 7 (DLEU7) expression in A549 lung cancer cells resulted in a decrease in the proportion of S-phase cells and increased apoptosis (8).

Several studies have focused on PON1 activity and genotype in various diseases. For example, PON1 has been identified as an independent risk factor for atherosclerosis. Serum paraoxanase 1 activity was significantly lower in patients with atherosclerosis (14). In addition, Serum PON1 activity was found to be significantly lower in patients with pancreatic (15) gastric (16) and lung (17) cancer when compared with healthy controls. A relationship between PON1 genotype and the risk of breast (18, 19) prostate (20) and lung (21) cancer, non-Hodgkin’s lymphoma (22) pancreatic (15) gastric (16) and lung (17) cancer, non-Hodgkin’s lymphoma (22) and other cancers has also been shown.

PON1 gene polymorphisms Q192R and L55M have been shown to be associated with several human cancer types, but their association with CML and CLL has yet to be investigated. In this study, we examined the PON1 192 and PON1 55 genotypes in relation to the risk of Turkish patients developing CML and CLL.

Materials and Methods

Patients. The study began by obtaining the approval of the Gaziantep University Ethics Committee (07-2007/40). The patient group consisted of 60 patients (33 males and 27 females; mean age of 48 years, range=18-78 years) with the diagnosis of or under treatment for CML and 60 patients (32 males, 28 females; mean age of 62 years, range=30-76 years) with the diagnosis of or under treatment for CLL at the Hematology Clinic, Gaziantep University Hospital. A total of 84 people (43 males, 41 females; mean age of 52 years, range=18-78 years) were included in the study as a control group, who were apparently healthy volunteers. No one in the control group had a smoking history or a chronic use of any drugs. Written-informed consent was obtained from the patients and controls after the explanation of the study details. After a 20-min rest, 2 ml blood samples were collected from the antecubital vein of each participant into tubes prepared with EDTA. All the blood samples were stored at −20˚C until DNA purification.

Determination of the PON1 192 gene polymorphism. Genomic DNA was extracted from mononuclear cells obtained from EDTA-treated peripheral venous blood using the salting-out method (25). The polymerase chain reaction (PCR) (25 μl) for the PON1 192 gene polymorphism was performed in 10 mM Tris-HCl, pH8.4, 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, 20 pmol of each primer (Biobasic, Ontario, Canada), and 1 U Taq polymerase (MBI Fermentas,Vilnius, Lithuania) in a thermocycler under the following conditions: 94˚C for 5 min (initial denaturation) followed by 35 cycles at 95˚C for 1 min (denaturation), 60˚C for 1 min (annealing), 72˚C for 1 min (extension), and a final extension at 72˚C for 10 min. A 99-bp fragment was amplified from 10 ng genomic DNA. The PCR products were restricted using restriction endonuclease A1wl (MBI Fermentas) and separated by 2% agarose gel electrophoresis. The PCR products of 99 bp were digested with 2U Alw1 at 55˚C for 20 h, and resulted in 99-, 69- and 30-bp fragments for the QR genotype, 69- and 30-bp fragments for the RR genotype, and a non-digested 99-bp fragment for the QQ genotype. The digested products were resolved by gel electrophoresis (2% agarose gel) and visualized by ethidium bromide staining.

Determination of the PON1 55 gene polymorphism. For the genomic DNA samples of the individuals, the alleles of the PON1 55 locus were amplified by PCR. The PCR (25 μl) was performed in 10 mMTris-HCl, pH8.4, 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, 20 pmol of each primer (Biobasic), and 1 U Taq polymerase (MBI Fermentas) mixed in a thermocycler under the following conditions: 94˚C for 2 min (initial denaturation) followed by 35 cycles at 94˚C for 1 min (denaturation), 60˚C for 1 min (annealing), 72˚C for 1 min (extension), and a final extension at 72˚C for 10 min. A 170-bp fragment was amplified from 10 ng genomic DNA. After the PCR amplification, a fragment belonging to the PON1 55 locus was digested by using Hsp92II (Promega, Madison, USA) a restriction endonuclease and separated in 2% agarose gel. The PCR products (170 bp) were digested with 2U Hsp92II at 37˚C for 20 h, and resulted in 170-, 126- and 44-bp fragments for the ML genotype, 126- and 44-bp fragments for the MR genotype, and a non-digested 170-bp fragment for the LL genotype. The digested products were resolved by gel electrophoresis (2% agarose gel) and visualized by ethidium bromide staining.

Statistical analysis. The analysis of the data was performed by using the computer software SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). The statistical significance of the differences between the patient and the control groups were estimated by logistic regression analysis. The adjusted odds ratios (ORs) were calculated with a logistic regression model that checked for gender and age, and reported with 95% confidence intervals (CI). Differences in PON1 allelic frequencies between the control group and patients were compared with the chi-square test, with the Fisher's exact test being used when required. The Hardy–Weinberg equation was used to calculate the estimated and observed genotypic
frequencies (26). A p-value of less than 0.05 was considered as statistically significant.

**Results**

The comparison of the Q/R192 polymorphism of the *PON1* gene between the patients with CML, CLL, and the healthy controls is shown in Table I. While the comparison of the patients with CML with the control group did not show any statistically significant relationship between the RR genotype or R allelic frequency and CML, both the RR genotype (p=0.04) and the R allele were statistically significantly more frequent in the CLL group (p=0.04). The comparison of the M/L55 polymorphism of the *PON1* gene between the patients with CML, CLL, and the healthy controls is shown in Table II. While the comparison of patients with CML with the control group did not show any statistically significant relationship for the genotypic and allelic frequencies, in CLL, the increase in the frequency of both MM (p=0.007) and a decrease in LL genotype (p=0.004) and in the R allele (p=0.001) were found to be statistically significant.

We did not observe any deviation from the Hardy–Weinberg equation in any of the groups (data not shown).

**Discussion**

Oxidative stress and free radicals have been associated with an increased risk in various types of cancers (27, 28). The PON gene family consists of three members, *PON1*, *PON2*, and *PON3*. *PON1* is found exclusively extracellular, and is associated solely with the high-density lipoprotein (HDL) particles in the circulation, and, in part, confers the anti-oxidant and anti-inflammatory properties associated with...
HDL (29). Our study shows that PON1 polymorphisms are risk factors in the development of CML and CLL exclusively.

In the present study, we did not find any significant relationship for the genotype nor the allelic frequency in patients with CML regarding the PON1 192 and PON1 55 polymorphisms. In a similar result, Rajaraman et al. did not find any relationship between brain tumors and the PON1 genotype (30). Akkiz et al. suggested that neither the Q192R polymorphism nor the L55M polymorphism have any relationship to the risk of developing hepatocellular carcinoma (1). Uyar et al. found that there were no significant differences in the L55M polymorphism and in L and M allelic frequencies in patients with renal cell carcinoma and those without. They concluded that the R allele may protect against renal cell carcinoma (31). Martinez et al. found that common non-synonymous PON1 polymorphisms are not related to the risk of developing astrocytoma and meningioma (32). Van Der Logt et al. found that PON1 does not contribute to the susceptibility to colorectal cancer (33).

In contrast to our findings, Stevens et al. suggested that PON1 L55 M allele may be associated with an increased risk of breast cancer in postmenopausal women (18). Deroo et al. found that the PON1 rs757158 polymorphism was associated with breast cancer (34). Conesa-Zamora et al. showed that PON1 GG genotype in rs662 polymorphism could be a risk factor for B-cell lymphoma (35). Vecka et al. suggested that PON1 plays an important role in pancreatic cancer, especially in patients with cancer-associated malnutrition (36). Marchesani et al. concluded that PON1 appears to be associated with an increased risk of prostate cancer (37). In another study, women carrying the PON1 55 L allele, compared with women with the AA genotype, had an increased risk of ovarian epithelial carcinoma (38).

In the present study, we found a statistically significant increase in frequency of both the RR genotype and the R allele for PON1 192, and an increase in the MM genotype, and a decrease in the LL genotype and R allele for PON1 55 in patients with CLL. Antognelli et al. showed that PON1 192 polymorphism, and the R allele were associated with a decreased risk of developing breast cancer, while polymorphisms in PON1 55 were associated with an increased risk of this neoplasia (39). Antognelli et al. found that PON1 192/QQ was associated with a significant increased risk of prostate cancer compared to the PON1 192/RR genotype (20). Ahmed et al. thought that the R allele may protect against colorectal cancer (40). In another study, Lee et al. showed that carriers of the PON1 192 QQ genotype have an increased risk of lung cancer (21). It was suggested in previous studies that the Q to R substitution leads to the production of the enzyme against carcinogenic products of oxidative stress (20, 41). This theory is confirmed by studies showing that individuals either had higher PON1 activity or had a higher frequency of R and L alleles (41, 42). Oztürk et al. concluded that QQ type PON1 enzyme activity may be protective against bladder carcinogenesis (43). Their findings support the notion that genotypic differences of PON1 might contribute to prognosis and pathogenesis of bladder cancer. Lee et al. found that PON1 polymorphism was associated with an increased risk of lung cancer. Individuals carrying the Q/Q genotype of the PON1 gene were found to be at higher risk of developing lung cancer (21). Eom et al. suggested that the protective effect of PON1 rs662 SNP against lung carcinogenesis, and the induction of oxidative stress, might be modulated by the interaction between PON1 genetic polymorphisms and tobacco smoking (44). Fang et al. suggested that the PON1 55M allele is a risk factor in the development of cancer, in particular of breast cancer (45). Arpaci et al. concluded that the PON1 192 AA genotype may play an important role as a risk factor for ovarian cancer in the Turkish population, and that the A and L alleles may be associated with the early onset of disease (46).

We did not find any significant relationship in the genotypic and allelic frequency in the CML group regarding the PON1 Q192R and PON1 M/L55 polymorphisms. We conclude that the R allele of PON1 Q192R gene polymorphism, and the MM genotype of PON1 M/L55 gene polymorphism play a role in CLL susceptibility, while the LL genotype may protect against CLL in the Turkish population. However, this is the first study on PON1 in CML and CLL, and study of a larger number of individuals is necessary.

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