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Investigation of GSTP1 (Ile105Val) Gene Polymorphism in Chronic Myeloid Leukaemia Patients

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ABSTRACT The factors leading to the development of Chronic Myeloid Leukemia (CML) are not fully known. Associations between polymorphisms for genes encoding Glutathione S-transferase (GST) enzymes involved in Phase II detoxification reactions and susceptibility to some cancers have been shown in several studies. The aim of the present study was to investigate the influence of the GSTP1 (IIe105Val) gene polymorphism on human susceptibility to CML. Seventy-one CML patients and 67 control subjects with no cancer history were enrolled in our study. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to identify the GSTP1 (Ile105Val) gene polymorphism. Genotypes were determined according to the bands that formed in agarose gels via gel electrophoresis. Leukocytes in the CML patient group were significantly different from those in the control group (p=0.0001). The frequency of the GSTP1 Val allele was found to be 22% in CML patients and 31% in controls. However, no statistical variation was found to exist between controls and patients in terms of the GSTP1 Val allele frequency (p=0.199). The relationship between the GSTP1 (IIe105Val) gene polymorphism and CML is not fully understood. Our results provide no evidence of a relationship between the GSTP1 (IIe105Val) gene polymorphism and susceptibility to CML in Turkish patients. However, these findings should be confirmed in studies with larger sample sizes.

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease resulting from neoplastic transformation of multipotent stem cells. The disease is characterized by high levels of leukocytes, splenomegaly, myeloid hyperplasia in bone marrow and high levels of mature myeloid cells in peripheral blood (Sawyers 1999; Kabarowski and Witte 2000; O'Dwyer et al. 2002). CML affects males more frequently than females and is generally observed in adults by their middle ages. While the factors that lead to the development of CML are not known, it is claimed that cytotoxic and genotoxic environmental agents (especially ionization, radiation and similar factors) may increase the risk of CML development (Taspinar et al. 2008; Ichimaru et al. 1978; Löffler et al. 2001).

Glutathione S-transferase (GST) enzymes, which are encoded by GST genes, are responsible for the detoxification of chemicals found in the environment and naturally synthesized metabolites, and they play an important role in protecting tissue from oxidative damage. Often, genetic polymorphisms within enzymes that play a role in the detoxification of xenobiotics are linked to an increase or decrease in the prevalence of certain cancer types in groups of individuals. A significant relationship is observed between the risk of developing cancer and genetic polymorphisms within enzymes involved with xenobiotic metabolism. This relationship has highlighted the role of genetics in cancer etiology (Kiran et al. 2010; Malats et al. 2000; Reszka et al. 2006).

It was discovered that one of the GST enzymes, GSTP1, is over expressed in various tumor types. It has been demonstrated that polymorphisms within the gene encoding GSPT1 (Ile105Val, Ala114Val) lead to reduced enzymatic activity and an inability to break down both chemotherapeutics and the carcinogens found in cigarettes. It was therefore claimed that variation in carcinogen breakdown among individuals forms a foundation for cancer development within humans (Taspinar et al. 2008; Sailaja et al. 2010; Strange et al. 2000; Armstrong 1991).

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To our knowledge, the GSTP1 (Ile105Val) gene has yet to be studied in Turkish CML patients. Thus, in this study, the researchers aimed to investigate the relationship between the development of CML, the etiology of which is not fully understood, and the GSTP1 Val allele, as well as determine the frequency of the Val allele in Turkish CML patients.

MATERIALS AND METHODS

Study Subjects

The study groups were established retrospectively by randomly selecting individuals from the group of patients who were registered in the Medical Genetics Department of the Medical School of Uludag University with a preliminary diagnosis of CML between the years of 2008-2009. In this study, the patient group consisted of 71 cases diagnosed with CML, and the age-matched control group consisted of 67 cases with no cancer history. For each individual in both the patient and control groups, all demographic data were recorded, including age, gender, leukocyte count and t(9;22) translocation results.

DNA Isolation and Genotyping of GSTP1 (Ile105Val)

Blood samples from both the patient and control groups were taken in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (Dr. Zeydanlý Life Sciences, Ltd., Turkey) according to the manufacturer's instructions, and samples were stored at -20 °C until performing PCR. The GSTP1 (Ile105Val) gene polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the GST-P1 polymorphism, forward 5'-ACCCCAGGGCTCT-ATGGGAA-3' and reverse 5'- TGAGGGCAC-AAGAAGCCCCT -3' primers were used (Abbas et al. 2004). To identify the GSTP1 (Ile105Val) gene polymorphism among the products, the Alw26 I (Genemark, Taiwan) enzyme was used. After digesting the enzyme in 4% agarose gel, the three possible genotypes of the GSTP1 allele were determined as follows: if the 176 bp PCR product from the GSTP1 gene was cut into two distinct products of 85 bp and 91 bp, then the GSTP1 allele genotype was identified as Val/Val; if the 176 bp PCR product resulted in three

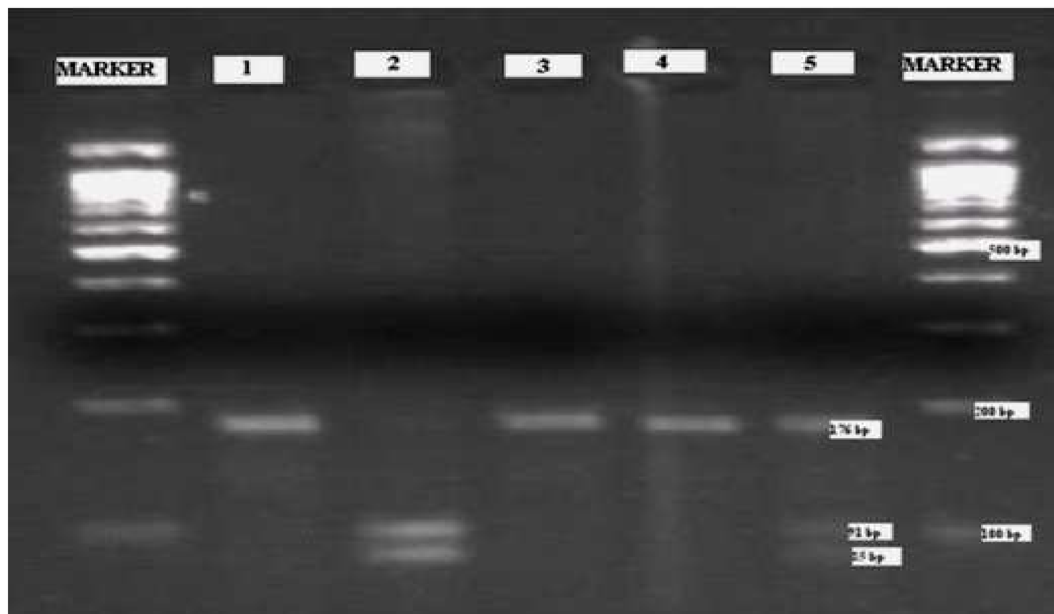

Fig. 1. Photograph of the PCR products of the GSTP1 gene after Alw 26 I enzyme digestion and on a 4 % agarose gel. Line MARKER shows the 100-bp DNA ladder; lines 1, 3 and 4 show individuals with the Ile/Ile genotype (176 bp); line 2 shows the Val/Val genotype (91 bp, 85 bp); and line 5 shows the Ile/Val genotype (176 bp, 91 bp, 85 bp)

distinct products of 176 bp, 91 bp and 85 bp, then the genotype was identified as Ile/Val; and if the 176 bp PCR product was the only product from the PCR procedure, then the genotype was identified as Ile/Ile (Fig. 1).

Statistical Analysis

Data were recorded in mean ± standard deviations. A chi-squared ($\chi 2$) test was used to compare genotypes. P values less than 0.05 were accepted as statistically significant.

RESULTS

The characteristics of the study population are shown in Table 1. The age distribution was not different between patients and controls, with the mean age being 49±12.84 and 49.86±10 years for patients and controls, respectively (p= 0.853). However, within the CML group, leukocyte counts were significantly different from those of the control group (p=0.0001).

Table 1: Characteristics and genotype distribution of the GSTP1 polymorphism in CML patients and control groups

Variables	*Patient group n=71*	*Control group n=67*	*p-value*
Age (years) (Mn±SD)	49± 12.84	49.86± 10	0.853
Gender			
Female	40	38	0.964
Male	31	29	
Leukocyte Count (Mn±SD)	129.590± 130.947	11.400± 5.142	0.0001
Genotypes			
Ile/Ile (A/A)	45	36	1(Reference)
Ile/Val(A/G)	21	21	0.822
Val/Val(G/G)	5	10	0.138
Frequency of Allele			
Ile (%)	78	69	1(Reference)
Val (%)	22	31	0.199

Mn: mean; SD: standard deviation

In terms of the GSTP1 (Ile105Val) polymorphism, it was observed that 45 CML patients had the Ile/Ile genotype, 21 had the Ile/Val genotype and 5 had the Val /Val genotype. In contrast, in the control group, 36 had the Ile/Ile genotype, 21 had the Ile/Val genotype and 10 had the Val /Val genotype. In comparing these two genotype distributions, no significant difference was found between CML patients and the control group (p>0.05) (Table 1). Overall, the frequency of the GSTP1 (Ile105Val) allele was observed to be 22% in the patient group and 31% in the control group. No significant differences were observed between the groups for the Val allele, although its frequency was higher in the control group (p=0.199) (Table 1).

The Val allele frequency in the control group used in the study conducted in Turkey is summarized in Table 2.

Table 2: Val allele frequency in control group of studies conducted with Turkish patients

Study	*The compared disease group*	*Number of control cases*	*Val allele frequency (%)*
Kiran et.al.	Cervical cancer	52	30
Tamer et al.	Gastric cancer	204	38
Yalin et al.	Type 2 Diabetes Mellitus	98	32
Balta et al.	Acute lymphoblastic leukemia	103	44
Altayli et al.	Bladder cancer	128	29
Aynacioglu et al.	Bronchial asthma	265	31
Calikoglu et al.	Chronic obstructive lung disease	150	65
This study	Chronic myeloid leukemia	67	31

DISCUSSION

Genetic variation influencing individual susceptibility to chemical carcinogens is one of the main factors leading to cancer development among human beings. Genetic variants within genes that encode enzymes involved with metabolism, such as CYP and GST, have been shown to increase the likelihood of developing various forms of cancers (Kiran et al. 2010; Idle 1991; Nebert 1991).

There are many studies that have investigated the relationship between GST polymorphisms and acute leukemia. In a review published by Ye and Song in which 30 published case-control studies were analyzed, it was observed that in particular, the GSTM1 and GSTT1 null genotypes were associated with an increased risk of developing acute lymphoblastic leukemia (ALL) (Ye and Song 2005). However, there is very little information on the role of GST polymorphisms in CML development. To the best of our knowledge, there are two studies in which GST polymorphisms and CML development

were analyzed, namely, those of Taspinar et al. and Sailaja et al. (Taspinar et al. 2008; Sailaja et al. 2010). In Taspinar et al.'s study, a group comprising 107 patients and 132 healthy controls was studied to analyze the relationship between CML development and the GSTM1 and GSTT1 polymorphisms. In this study, the GSTT1 homozygous deletion (null) genotype was determined to be higher in the patient group (40.2%) than in the control group (19.2%), and it was hypothesized that the null genotype may be a protective factor against the development of CML. However, there was only a small increase in the frequency of the GSTM1 null genotype in the patient group compared with the patient group, and no significant difference was detected (Taspinar et al. 2008).

Sailaja et al. used an experimental design similar to the researchers to study the GSTP1 (Ile105Val) polymorphism in a group consisting of 260 patients and 248 healthy controls. They analyzed the development of the disease and its response to treatment. In terms of response to treatment, they observed no statistically significant difference. However, when comparing GSTP1 Val/Val genotype frequencies between groups, a significant difference was observed, with genotype frequencies of 6.5% in the CML group and 1.2% in the control group ($p = 0.0084$). In determining significant variation between CML development and the GSTP1 (Ile105Val) polymorphism, it was reported that the Val/Val genotype increased the risk of CML development by changing GSTP1 enzyme activity (Sailaja et al. 2010). However, when the allele frequency was analyzed, very similar values were obtained between the two groups, with frequencies of 26% in the patient group and 24% in the control group.

Although no significant difference is observed between the groups in terms of age and gender, a significant difference was observed in terms of leukocyte number ($p=0.0001$). Moreover, when analyzed in terms of the GSTP1 (Ile105Val) polymorphism, the Val allele frequency was observed to be 22% in the patient group and 31% in the control group. Although the Val allele frequency was higher in the control group, no significant difference was determined ($p=0.199$). In terms of the frequency of the Val allele, our results are extremely similar to those of Sailaja et al. However, when the genotype distribution is analyzed, our results are contrary to the study of Sailaja et al. and no statistically significant difference between case and control groups was determined ($p> 0.05$). Because our study is limited in terms of the number of patients studied, other studies need to be conducted that include cases from a wider spectrum of different geographical areas and ethnic groups.

In this study, in addition to analyzing the relationship between the GSTP1 (Ile105Val) polymorphism and CML, the researchers also investigated the Val allele frequency within control groups of studies conducted with Turkish patients. The reason they conducted these analyses was to investigate the probability that gene polymorphisms may vary among different human ethnic and geographic backgrounds. In studies conducted with CML rates similar to this study, Val allele frequencies have generally been determined to be 30-45%, and only in the study conducted by Calikoglu et al. is the rate determined to be at a greater frequency, 65% (Kiran et al. 2010; Tamer et al. 2005; Yalin et al. 2007; Balta et al. 2003; Altayli et al. 2009; Aynacioglu et al. 2004; Calikoglu et al. 2006). However, the Calikoglu et al. study reported the Val allele frequency in patients to be 35 %.

To conclude, in this study, the researchers have investigated the relationship between CML and the GSTP1 (Ile105Val) polymorphism. Information gained from studies conducted on this issue may guide us in the diagnosis and treatment of this disease as well as protection against developing it. In this study, while no significant relationship was detected between CML and the GSTP1 (Ile105Val) polymorphism, the number of cases used in this study was smaller than those in other polymorphism studies. Thus, the researchers have concluded that this study will need to be supplemented by others that include a wider spectrum of cases from different geographical and ethnic backgrounds. Additionally, other studies will need to be conducted to investigate the relationship between other single nucleotide polymorphisms within the GST gene and CML.

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