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Association of Acute Lymphoblastic Leukemia and MDR1 Gene Polymorphism in an Ethnic Iranian Population

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Abstract

Background: The frequency of the multi-drug resistance 1 (MDR1) gene C3435T polymorphism differs in various ethnical populations such as Asian, African, and Caucasians populations. A silent C3435T polymorphism in exon 26 of MDR1 has been reported to be associated with a decreased expression of P-gp in TT genotypes carriers compared with CC genotypes carriers.

Materials and Methods: To evaluate the association between MDR1 gene C3435T polymorphism and acute lymphocyte leukemia (ALL), 126 ALL patients (72 males and 54 females) with a mean age of 11.42 ± 6.55 and 139 healthy controls (79 males and 60 females) with a mean age of 12.15 ± 7.5 who were referred to Dr. Sheykh hospital, Mashhad, Iran, between 2005-2007 were enrolled in our study and their C3435T MDR1 polymorphism was investigated using PCR-RFLP.

Results: The mutant homozygous TT and TC genotypes were found to be associated with the incidence of ALL (p<0.05). There was no significant difference for T allele frequency between ALL patients and healthy controls (OR=1.08, 95% CI; 0.84-1.66, p=0.33).

Conclusion: TT genotypes carriers are at higher risk of developing ALL than carriers of other genotypes.

Keywords: p-glycoprotein, multi-drug resistance, acute lymphoblastic leukemia, polymorphism

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and represents 20% of acute leukemias in adults.[1,2] Eighty percent of children with ALL have a good prognosis for remedy. In adults, there is a high remission rate after the initial treatment; however, only 20%-40% of patients achieve long–term survival.[1] Little is known about the pathogenesis of ALL, although both inheritance and specific environmental exposures are supposed to play a role in this process. The genetic polymorphisms, which determine differences in the activity of enzymes involved in transport and metabolism of mutagens (e.g. glutathione S–transferase or cytochrome family genes) are promising areas to search for risk factors of developing ALL.[3]

P-glycoprotein (P-gp), the product of multidrug resistance 1 gene (MDR1), is an important ATP-dependent membrane transporter which is involved in the absorption, distribution, and elimination of numerous drugs and acts as

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energy–dependent efflux pump that exports its substrates out of the cell.[4,5] The most important role of P-gp is the protection of the organism against xenobiotics and toxic compounds.[3] P-gp expression in tumor cells is associated with multidrug resistance phenotype in some hematological malignancies, e.g. acute myeloid leukemia (AML) or adult ALL. Anthracylines, vinca alkaloids and epipodophyllotoxins, which are crucial drugs in the chemotherapy of ALL, are P-gp substrates.[2,6-9] At least 28 single nucleotide polymorphisms of MDR1 gene locus have been reported.[3,10-13] Hoffmayer et al.[13] have reported a silent polymorphism, which is associated with the expression of P-gp. This polymorphism consists of a C to T exchange at position 3435 in exon 26 of the MDR1 gene. Individuals with the 3435TT genotype have significantly lower duodenal MDR1 expression and function than those with the 3435CC genotype.[12,14] It is supposed that the lower expression of P-gp can cause accumulation of xenobiotics and toxic compounds in the cell and results in predisposition to diseases such as cancers. The allelic frequencies of the MDR1 gene C3435T differ in various populations so we aimed to evaluate the association between MDR1 gene polymorphism with the incidence of ALL in an ethnic Iranian population.

Materials and Methods

Subjects

126 patients with ALL and 139 healthy controls of Iranian origin were enrolled in our study. All patients were referred to Dr. Sheykh hospital, Mashhad University of Medical Sciences, Mashhad, Iran, between May 2005 and October 2007. The study was approved by the Ethical Committee of Mashhad University of Medical Science. All subjects gave their informed consent.

One of the patients had also been diagnosed with cerebral palsy and one with Down's syndrome. The control group consisted of volunteers who had attended the hospital for blood sampling for biochemistry and/or hematologic analysis, and those who were willing to participate in the study. Subjects with any hematologic or other malignancies were excluded. We grouped together all infants, children, and adults with ALL because we found no statistical difference in the incidence of polymorphism between patients aged 20 years or younger (infant and childhood ALL) and those older than 20 (adult ALL).

Genotyping

Whole blood was collected from the enrolling subjects and genotyping of ALL patients and healthy controls was performed by Polymerase Chain reaction–Restriction Fragment Length Polymorphism (PCR-RFLP).

DNA was isolated from peripheral blood cells using a standard salting out protocol. The C3435T variant of the MDR1 gene was identified with primers: 5’-ACT CTT GTT TTC AGC TGC TTG-3' as the forward and 5'-AGA GAC TTA ACT TAG GCA GTG ACT-3' as the reverse primer, yielding the 206-base-pair (bp) product. The primer design was based on the published sequences for genotyping procedure of MDR1 polymorphism using genomic DNA.[3] For PCR reactions, 200 ng of genomic DNA was amplified in 50 µL of reaction mixture containing 200 µM of each of dNTPs (dATP, dCTP, dGTP and dTTP), 250 ng of each primer, 1.5 mM MgCl2 and 1 U Taq DNA polymerase (MBI Fermentas). PCR amplification consisted of an initial 5-minute denaturation at 94˚C, followed by 35 cycles of denaturation at 94˚C for 90 seconds, annealing at 60˚C for 30 seconds, and extension at 72˚C for 30 seconds. The terminal extension was performed at 72˚C for 10 minutes. Amplified DNA fragments (206 bp) were digested by MboI enzyme (Fermentas) for 24 h in 37˚C. The PCR product was identified in a 1% (w/v) agarose gel stained with ethidium bromide and visualized directly under UV light.

Statistical analysis

Statistical analysis of the data was performed by SPSS 11.5 software and chi-square tests. Odd ratio (OR) with 95% confidence interval (CI) was calculated.

Results

The ALL patients (1-63 years old, 72 males (57.1%), 54 females (42.9%), with a mean age of 11.42 ± 6.55) and the control group (1-68 years

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Table 1: *Frequencies of genotypes in ALL patients and the control group in the MDR1 study*

	Genotypes					
	TT	**TC/CC**	**TC**	**TT/CC**	**CC**	**TT/TC**
Patients (n=126)	34 (27.0%)	92 (73.0%)	73 (57.9%)	53 (42.1%)	19 (15.1)	107 (84.9%)
Control (n=139)	22 (15.8%)	117 (84.2%)	100 (71.9%)	39 (28.1%)	17 (12.2%)	122 (87.8%)
P- value	0.026		0.017		0.499	
OR (95% CI)	1.96 (1.07-3.58)		1.96 (1.07-3.58)		1.96 (1.07-3.58)	

Table 2*: The comparison of TC and TT genotypes in ALL patients and control group in the MDR1 study*

Genotype	Patients	Control	Total	P-value	* OR (95% CI)
TT	34 (31.8)	22 (18.0)	56 (24.4)	0.016	OR = 2.117, (1.144-3.917)
TC	73 (68.2)	100 (82.0)	173 (75.6)		

*Confidence Interval

old, 79 males (56.8%), 60 females (43.2%), with a mean age of 12.15 ± 7.5) consisted our 265 subjects in this study. In vitro DNA amplification of the MDR1 gene using the specific primers resulted in a 206 bp DNA product. Digestion of amplified fragment (amplicon) with MboI restriction endonuclease resulted in DNA fragments of 130-bp (CC); 206-bp (TT); or 130 and 206-bp (TC). The mutant homozygous TT genotype and heterozygous CT genotype were found to be significantly associated with the occurrence of ALL (OR=1.96, 95% CI; 1.07-3.58, p=0.026 for TT genotype and OR=1.96, 95% CI; 1.07-3.58, p=0.017 for TC genotype, Table 1). The risk of developing ALL in carriers with TT genotype was 2.1 folds higher compared to carriers of TC genotype (p=0.016, OR, 95% CI; 2.117, Table 2). The allelic frequencies for C and T alleles were 44% and 56%, respectively. There was no significant difference for C and T allele frequency between ALL patients and healthy controls (OR=1.18, 95% CI; 0.84-1.66, p=0.33, Data not shown). Moreover, there was no significant association between MDR1 gene C3435T polymorphism and clinical parameters including age at the time of diagnosis and sex (p=0.76, Data not shown).

Discussion

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and represents 20% of acute leukemia in adults. We found an about 2-fold (1.96) increase in the risk of developing ALL in TT genotype carriers (p=0.026, Table 1). We also found an interesting significant difference in TC genotype carriers between patients with ALL and healthy controls (p=0.017). Incidence of ALL in TC genotype carriers compared to TT genotype carriers showed an about 2-fold (2.117) decrease. It may be because of the protective role of C allele in a way that even one allele of C can decrease the incidence of ALL to half. As described above, we grouped together all infants, children, and adults with ALL. It is suggested that TT genotype may be associated with the incidence of ALL more than TC genotype (Table 2). There was a significant difference between TT and TC genotypes (p=0.016). On the other hand, there was no significant difference for C and T allele frequency between ALL patients and healthy controls. Many studies did not found any significant association between MDR1 gene C3435T polymorphism and the incidence of diseases or cancers. One study reported that the single nucleotide polymorphisms considered individually or within haplotype C3435T was not significantly associated with childhood ALL.[15] Urayama et al. (2002) did not find any significant difference in C3435T gene MDR1 polymorphism between patients and controls.[16] This may be because they did not stratify patients in AML and

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ALL as well as in Hispanic and non- Hispanic populations. The genotype frequency distribution of MDR1 CC, CT and TT genotypes in Tamilian (South Indian) population was 18%, 56%, and 25%, respectively, whereas the allel frequencies were 46% and 54% for C and T alleles, respectively.[17]

Our study is in accordance with Jamroziac et al. (2004) findings on the association between MDR1 gene C3435T polymorphism and ALL in children. They found that TT genotype was associated with the occurrence of ALL (OR=1.80, 95% CI; 1.10-3.10, p=0.037.[3] Hoffmayer et al. (2000) also demonstrated the contribution of MDR1 gene in the pathogenesis of inflammatory and malignant disorders of the gasterointestinal tract. They reported that the MDR1 gene C3435T polymorphism was a silent polymorphism.[13] The reason why this polymorphism can influence the function and expression of P-gp remains unanswered. It may be in linkage disequilibrium with other polymorphism(s). A polymorphism at position 2677 in exon 21 of the MDR1 was found to co-segregate with C3435T in some studies.[10,18,19]

In conclusion, we found that T- allele carriers have an increased risk of developing ALL when compared to other subjects. More studies with higher number of patients are needed to elucidate the association between C3435T polymorphic variant forms with ALL.

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