ORIGINAL ARTICLE

Association between *MDR1* C3435T Gene Polymorphism and Acute Lymphoblastic Leukemia (ALL) in Iranian Population

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Abstract

Background: P-glycoprotein (P-gp), an ATP-dependent efflux pump, is a membrane protein encoded by *MDR1* gene. P-gp has an important role in protection of the cell against xenobiotics and toxic compounds. Recently, 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.

Methods: To evaluate the distribution of allelic variants of C3435T *MDR1* in a group of healthy population in Iran and find the association between *MDR1* C3435T polymorphism and the incidence of ALL, 126 patients with ALL and 139 healthy controls were included in our study and their *MDR1* polymorphisms were detected by PCR-RFLP assay.

Results: 71.9% of the healthy people had 3435TC genotype, 15.8% had 3435TT genotype and 12.2% had 3435CC genotype. Also, the frequency of T allele was 51.8% and C allele 48.2%. The mutant homozygous TT and TC genotypes were found to be associated with the incidence of ALL (OR=1.96 for TT genotype and OR=0.53 for TC genotype).

Conclusion: *MDR1* C3435T polymorphism may contribute to the incidence of ALL. TT genotypes carriers are more at risk of developing ALL than other genotypes carriers.

Keywords: P-glycoprotein; *MDR1*; Acute lymphoblastic leukemia; C3435T 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 the 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 the patients achieve long term survival.[1,2] Little is known about pathogenesis of ALL, although both inherited and specific environmental exposure is 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 a promising area to search for risk factors of developing ALL.[3]

P-glycoprotein(P-gp), the product of multidrug resistance gene (*MDR1*), is an important ATP- dependent membrane transporter which is involved in the absorption, distribution and elimination of numerous drugs and acts as an energy–dependent efflux pump that exports its substrates out of the cell.[4,5] The most

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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 epipodophillotoxins, 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] Hoff Mayer *et al*. (2000) have reported a silent polymorphism, which is associated with the expression of P-gp.[10] This polymorphism consists of a C to T exchange at position 3435 in exon 26 of the *MDR1* gene. Individuals with the 3435TT genotype had significantly lower duodenal *MDR1* expression and function than those with the 3435CC genotype.[10,13] 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. Although ethnic variation has been observed for most SNPs, the population frequency of C3435T has not been evaluated. Evaluation of *MDR1* genotype status may be a valuable tool in identifying individuals who may have altered drug absorption or are at higher risk for clinically significant drug interactions.[14] One promising possibility to optimize the efficacy and toxicity of cytotoxic treatment is a concept of the individualized chemotherapy. The term refers to the individual drug choice and dose adjustment according to pre-treatment analysis of natural genetic polymorphisms affecting drug transport and metabolism.[1,15] Therefore, there was an attempt to evaluate *MDR1* C3435T gene polymorphism in a group of healthy Iranian population. We also aimed to evaluate the association between *MDR1*C3435T polymorphism and the incidence of ALL.

Materials and Methods

One hundred and twenty six patients with ALL and 139 healthy controls of Iranian origin (Mashhad) were enrolled after being informed about the research and giving their consent to participate in the research. The blood samples were collected from the ALL patients who referred to Dr. Sheykh Hospital, a center for ALL patients, affiliated to Mashhad University of Medical Sciences.

The median age (1-63 y) was 11.42 years with an SD of 9.8. The patients were divided into two groups of children and adults. The children were divided into two groups of 1-9 y and 10-19 y. All the adults were ≥ 20 years old. However, among the patients, one had been diagnosed with cerebral palsy and one with Down's syndrome. Stratification for clinical factors was done for age, sex, and white blood cell count (WBC) at diagnosis. Whole blood was collected from the enrolled 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. For a 50 µL PCR, the reaction contained 200 ng genomic DNA, 200 µmol l^{-1} of each of dNTPs (dATP, dCTP, dGTP and dTTP), 250 ng of each primer (5'-ACT CTT GTT TTC AGC TGC TTG-3' and 5'-AGA GAC TTA ACT TAG GCA GTG ACT-3'), 1.5 mmol l^{-1} magnesium chloride and 1 U *Taq* DNA polymerase (MBI Fermentas). The primer design was based on the published sequences for genotyping procedure of *MDR1* polymorphism using genomic DNA.[3] PCR amplification consisted of an initial 5 min denaturation at 94°C, followed by 35 cycles of denaturation at 94˚C for 90 S, annealing at 60˚C for 30 S, and extension at 72˚C for 30 S. Amplified DNA fragments (206 bp) were digested by *Mbo*I enzyme (Fermentas) for 24h in 37˚C. After digestion, DNA fragments of 130 and 76 bp for homozygous wild type C allele or one 206 bp band for homozygous mutant T allele, and three bands of 206, 130 and 76 bp for heterozygous CT genotype were expected. Statistical analysis of the data was performed by SPSS software. To evaluate the relationship between the occurrence of ALL according to various genotypes, student t test was carried out to find any difference between the control group and the cases with the various genotypes such as TT, TC, CC (each separately). Chi Square test was used tor compare TT and TC genotypes, and also T and C alleles; odd ratio (OR) with 95% CI was calculated when $p<0.05$.

Results

DNA was extracted from whole blood and the fragments were amplified. The DNA was then digested with *Mbo*I enzyme (Figure 1). The genotypes frequency distribution were determined in our 139 healthy people that were considered as control and found 15.8%, 71.9%, 12.2% for TT, TC and CC genotypes, respectively. T and C alleles' frequency distribution were evaluated in the subjects and found 51.8% for T allele and 48.2% for C allele.

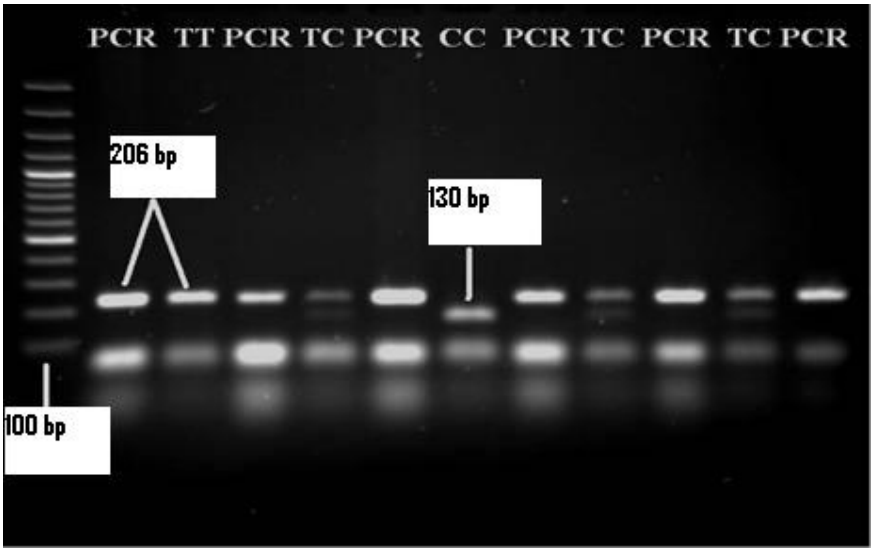

Fig. 1: Polymorphism of the *MDR1* gene. DNA ladder marker in left lane is labeled. The photo shows genotypes obtained from study cases TT (206) and CC (130) seen on the ethidium bromide-stained gel. These patterns were observed in both control and patient groups with the frequencies reported in Tables 1, 2 and 3. The homozygous (TT) has a band of 206 bp, the homozygous (CC) has a band of 130 bp and the heterozygous (AC) has two band (not shown).

The mutant homozygous TT genotype and heterozygous CT genotype were found to be significantly associated with the occurrence of ALL (p=0.026, OR, 95% CI; 1.96, for TT genotype and p=0.017, OR, 95% CI; 0.53 for TC genotype) (Table 1). The risk of developing ALL in carriers with TT genotype was 2.1 fold compared with carriers of TC genotype (p=0.016, OR, 95% CI; 2.117) (Table 2). Also, higher T allele frequency in ALL patients was observed when compared with healthy controls although this difference was not significant (p=0.338) (Table 3). The results of PCR and RFLP are shown in Figure 1. Moreover, the association of *MDR1* gene C3435T polymorphisms with clinical parameter in-

Table 1: The genotypes frequency distribution in groups

Genotype	Patients (n=126)	Control (n=139)	Total (n=265)	*P* value	* OR (95%CI)
	No. (%)	No. (%)	No. (%)		
TT	34.0 (60.7)	22.0 (39.3)	56.0 (100.0)	0.026	OR=1.96, (1.07-3.58)
TC/CC	92.0 (44.0)	117 (56.0)	209 (100.0)		
TC	73.0 (42.2)	100 (57.8)	173 (100.0)	0.017	OR=1.96, (1.07-3.58)
TT/CC	53.0 (57.6)	39.0 (42.4)	92.0 (100.0)		
CC	19.0 (52.8)	17.0 (47.2)	36.0 (100.0)	0.499	OR=1.96, (1.07-3.58)
TT/TC	107 (46.7)	122 (53.3)	229 (100.0)		

*Confidence interval

Table 2: The comparison of TC and TT genotypes in groups

Genotype	Patients	Control	Total	*P* value	* OR (95%CI)
	No. (%)	No. (%)	No. (%)	0.016	OR = 2.117, (1.144-3.917)
TT	34.0 (60.7)	22.0 (39.3)	56.0 (100.0)		
TC	73.0 (42.2)	100 (57.8)	173 (100.0)		
Total	107 (46.7)	122 (53.3)	229 (100.0)		

*Confidence interval

Table 3: T and C alleles' frequency distribution in groups

Allele	Patients No. (%)	Control No. (%)	*P* value	* OR (95%CI)
T	141 (49.5)	144 (50.5)	0.338	OR=1.18, (0.84-1.66)
C	111 (45.3)	134 (54.7)		

*Confidence interval

cluded WBC and age at diagnosis and sex while no significance association was found among different genotypes carriers.

Discussion

In our study, 71.9% of the healthy people had TC genotype of *MDR1* C3435T polymorphism that is an intermediate form for expressing P-gp according to Hoffmayer *et al's.* (2000) study. The frequency of allele C was 48.2% and although it is lower than T allele, it is almost at equal frequency (51.8%). Some of the studies have detected the frequencies of population's polymorphisms. Ameyaw *et al.* (2001) detected the frequencies in Africans such as Ghanian, Kenyan, African American and Sudanese and found 83%, 83%, 84%, 73% for C allele in these populations respectively and the British Caucasian, Portuguese, South-west Asian, Chinese, Filipino and Saudi populations had lower frequencies of the C allele compared to the African group (48%, 34%, 53%, 59% and 55%, respectively).[14] Allele frequencies in our study proved to be similar to British Caucasian populations. These results give basis for large-scale investigations of *MDR1* C3435T genotype phenotype correlation in Iranian population that may be useful for designing individual-specific therapy for diseases like cancers, HIV-I infection and some other diseases.

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and represents 20% of acute leukemias in adults. We found around 2 (1.96) fold increase in the risk of developing ALL in TT genotype carriers (Table 1). We also found an interesting significant difference in TC genotype carriers between patients with ALL and the healthy controls. The incidence of ALL in TC genotype carriers compared with that in TT genotype carriers showed about 2 (2.117) fold decrease (Table 2). It may be due to the protective role of C allele in such a way that even one allele of C can decrease the incidence of ALL to half. According to Table 2, it is suggested that TT genotype may be more associated with the incidence of ALL than TC genotype. There was a significant difference between TT and TC genotypes (p=0.016). On the other hand, the frequency of T allele was higher in patients with ALL than C allele, although the difference was not significant (Table 3). Many studies did not find any significant association between *MDR1* gene C3435T polymorphism and the incidence of diseases or cancers. Urayama et al. (2002) did not detect any significant differences in C3435T gene *MDR1* polymorphism between the patients and controls. This may be because they did not stratify patients in AML and ALL as well as in Hispanic and non-Hispanic populations.[16]

Our results are in accordance with Jamroziac *et al*.'s (2004) findings on the association between *MDR1* gene C3435T polymorphism and ALL in children. They found that TT genotype was associated with occurrence of ALL (OR, 95%; 1.8, 1.1 – 3.1). Hoffmayer *et al.* (2000) also found the contribution of the *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. The reason of how 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.[17-19] Zheng *et al.* (2002) found that the *MDR1* C3435T genotype is associated with the G2677 genotype in pediatric heart transplant patients.[20] In conclusion, we found that the T- allele carriers have an increased risk of developing ALL when compared with the other carriers. Any way more studies are needed to detect the association.

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Conflict of interest: None declared.

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