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Original Articles

Evaluation of C3435T *MDR1* Gene Polymorphism in Adult Patient with Acute Lymphoblastic Leukemia

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Abstract:

Background: *P-glycoprotein (P-gp) is a transmembrane pump encoded by* MDR1 *gene. It contributes in protection of the cells against xenobiotic and toxic compounds. P-gp also contributes in multidrug resistance in acute lymphoblastic leukemia (ALL). Human* MDR1 *polymorphism include C to T exchange at position 3435, individuals with TT polymorphism have lower expression of P-gp than the others with CC genotypes. Accumulation of xenobiotics in the cells can cause some diseases like cancers.*

Materials and Methods: *To evaluate* MDR1*C3435T gene polymorphism in adult patients with ALL, 54 patients and 96 healthy controls were involved in our survey. Genotyping of ALL patients and healthy controls was performed by Polymerase Chain Reaction – Restriction Fragment- Length Polymorphism (PCR-RFLP). Data analysis was done by SPSS software through T-test and Chi- Square.*

Results: *No significant difference was found between C3435T* MDR1 *gene polymorphisms and incidence of ALL in adult patients. Also there was not any significant difference between T and C alleles and incidence of ALL.*

Conclusion: *C3435T* MDR1 *polymorphism is not associated with the incidence of ALL in the population studied.*

Keyword: *P-gp, C3435T,* MDR1*, ALL, polymorphism*

Introduction:

Acute lymphoblastic leukemia (ALL) is considered as a clonal lymphoblastic disease that includes about 20% of adult acute leukemias. At present, its treatment is based on intensive, multi – agent chemotherapy. However, prognosis of adult ALL is not yet very clear. Despite high remission rate after the primary treatment, relapses are very usual which is approximately about 20 – 40 % of patients who able to survive for long- term.[1,2] Moreover, intensive chemotherapy is often associated with significant side effects, especially in older patients.[3] Therefore, unsatisfactory long term efficacies of chemotherapy and treatment toxicity are major problems to be faced by the therapeutic developments in adults ALL.[4] One promising possibility to optimize efficacy and toxicity of cytotoxic treatment is a concept of the individualized chemotherapy.[2,5] 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.[2,5] In this context, the multi drug resistance – 1 (*MDR1*) gene, also known as adenosine triphosphate – binding cassette subfamily B transporter – 1 (ABCB1), is one of the most interesting candidates for pharmacogenetic studies.[6] *MDR1* gene encodes P – glycoprotein (P –gp), which belong to the family of ABC transporter proteins. P–gp is a membrane-associated protein, which acts as an ATP – dependent pump taking part in the transmembrane transport of many xenobiotics, including steroid, anthracyclines and Vinca alkaloids that are important in chemotherapy of adult ALL.[7,8] The functional role of P – gp as an efflux pump has been well established, relating to drug transport across cellular membranes, thus preventing their intracellular accumulation. Differences in the P –gp activity may reflect genetic polymorphism.[7,8] Human *MDR1* polymorphism has been described and correlated with potential clinical effects. This polymorphism is a C to T exchange at position 3435 T polymorphism significantly correlated with changes in the expression and activity of P –

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gp.[9] Individuals with the T/T genotype had significantly lower duodenal *MDR1* expression than those with the C/C genotype. The difference in P–gp levels between two groups was greater than 65–fold, and affected the pharmacokinetics of the glycoprotein substrate, i.e. digoxin.[9] Previous studies showed a predisposing role for *MDR1* C3435T gene polymorphism in children with ALL.[10,11] 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. On the other hand higher expression of P-gp, in the patients, may worsen their prognosis because of its functional effects in effluxing chemotherapeutic agents from the cell membrane. Accordingly, we tried to evaluate C3435T *MDR1*gene polymorphisms in adult patients with ALL.

Materials and Methods

54 patients with ALL and 96 healthy people comprising the control group -of Iranian origin- participated in our study. All patients were referred to Vali-e-Asr Hospital, affiliated to Fasa University of Medical Sciences, Fasa, Fars, Iran. After explaining the objectives of the research program to the subjects and obtained written permission from them, we took the subjects' demographic information including name, age, sex, living area, family history of cancer (first and second generation). The patients with other types of cancer and those below the age limit (≥ 20 years) of the study were excluded. The control group was chosen from healthy people with corresponding demographic information were referred to the hospital lab for the check up. Then, they were asked if there was any history of family cancer in their first and second generation. If they had, they would have been excluded from the study.

Three ml 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. For a 50µl PCR, the reaction contained 200 ng genomic DNA, 200 mmol/l 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', (Microsynth, Swiss)], 1.5 mmol/l magnesium chloride and 1 U *Taq* DNA polymerase (Fermentas, Litwini). The primer design was based on published sequences for genotyping procedure of *MDR1* polymorphism using genomic DNA (10). PCR amplification consisted of an initial 5 min denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for90 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, USA) 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 the SPSS software.The Chi-square tests, odd ratio (OR) with 95% CI was calculated when $P < 0.05$.

Results:

54 patients with ALL of both sexes (29 male, 25 female) and 96 healthy controls (49 male, 47 female) of Iranian origin participated in our study. The mean age in the two groups was almost the same (41±6.22 for patients and 44±5.85 for the control group). The clinical factor like white blood cell count (WBC ≥30000, <30000) at diagnosis were measured. We found 18.5% of TT genotype and 59.30% of TC genotype and 22.20% of CC genotype among patients with ALL. However, the control group showed 16.7% of TT genotype and 62.5% of TC genotype and 20.80% of CC genotype (Table-I). DNA was digested with *Mbo*I enzyme and DNA fragments detected after electrophoresis. No significant difference was found among different genotypes in groups after PCR-RFLP (Table-II). We found no significant difference between two groups alleles (P = 0.969). Moreover, the association of *MDR1* gene C3435T polymorphism with parameters included WBC at diagnosis and sex were evaluated. However, no significant difference was found between these parameters and different genotypes (P = 0.39, P = 0.99 respectively).

Table-I

The genotypes frequency distribution in groups

Groups	Genotypes				Alleles		
	TT N (%)	TC N (%)	CC N (%)	Total N (%)	C N (%)	T N (%)	Total N (%)
Patient	10(18.50)	32(59.30)	12(22.20)	54(100)	56	52	108
Control	16(16.70)	60(62.5)	20(20.80)	96(100) 100	92	192	
Total	144	156	150(100)	32(21.30)	92(61.30)	26(17.30)	

According to chi-square test there was not any significant difference between T and C alleles in cases and controls (p = 0.969)

Table-II

Genotype frequency distribution in groups

Genotype	Case (n=54)	Control (n=96)	Total (n=150)	P-value	OR(95% CI*)
	Number (percent)	Number (percent)	Number (percent)		
TCTT/CC	32(34.80)22(37.90)	16(61.50)80(64.50)	26(100)124(100)	0.774	1.136(0.475-2.717)
TTTC/CC	10(38.50)44(35.50)	60(65.20)36(62.10)	92(100)58(100)	0.696	0.873(0.441-1.726)
CCTT/TC	12(37.50)42(36.70)	20(62.5)76(63.3)	32(100)118(100)	0.842	1.086(0.484-2.438)

*Confidence Interval

Discussion:

We showed in our previous study (2010) a predictor role for C3435T *MDR1* gene in children with ALL, 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), (11). This study showed that C3435T polymorphism of *MDR1* gene is not a major predictor for ALL in adults. No significant association between C3435T polymorphisms of *MDR1* gene and incidence of ALL was found (Table-II). Frequency distribution of T and C alleles in patients and healthy controls did not show any significant differences too (P = 0.969). We also analyzed the WBC at diagnostic in patients. The results showed that there was not any association between different genotypes and WBC (P=0.39). However, the different genotypes and sex differences also did not show any significant changes among the patients (P = 0.99). Such a significant difference is due to various effective variables on the ALL incidence in adults as well as children. Significantly increased frequencies of the 3435T allele and the 3435TT were observed in patients with colorectal cancer compared with controls (P = 0.03; OR, 95% CI; 1.46 for 3435T allele and P = 0.003; OR, 95% CI; 2.2 for 3435TT genotype) in a research by Khedri et al.[12] There is a report which shows no significant association between different genotypes and ALL in adults (Jamroziak et al. 2005), though their patient sample size was 44.[1] There are several reports which showed no significant differences between C3435T *MDR1* gene polymorphisms and other diseases, In the study done by Tatari et al., it was shown there were no significant differences in genotypes and allele frequencies between patients with breast cancer and control subjects in an Iranian population.[13] Kim et al. suggested C3435T polymorphism in the multidrug-resistant epilepsy may not be significant in Korean populations.[14] However, the association of C3435T*MDR1* gene polymorphism with incidence of different diseases is yet controversial. The question of how C3435T polymorphism can effect on ALL blasts is not yet answered by any report to the best of our knowledge. As C3435T polymorphism is a silent mutation and it can not affect on amino acid sequences encoding protein, it is impossible that this polymorphism can affect P-gp expression directly, but it is clear that it has functional results; this single nucleotide polymorphism (SNP) is associated to an unknown factor. However Kimchi et al showed a silent mutation in a complex, that alters the substrate specifity in mammalian membrane transport protein, when frequent codons are changed to rare codons in a cluster of infrequently used codons, the timing of cotranslational folding is affected and may result in altered function.[15] Interestingly there is an intensive association between C3435T and G2677 (A, T) polymorphisms. As G2677 (A, T) polymorphism is a deletion mutation, it may have affects on activity and expression of P-gp, so the genotypes in C3435T or 2677 locations might help and lead us to evaluate *MDR1* function. Some other reports showed an association between *MDR1*C3435T gene polymorphism and expression of CYP3A4 (cytochrom P450).[1,16] Therefore, we suggest that it is important to consider these factors in future studies. Our study concludes that C3435T *MDR1* polymorphism is not associated with the susceptibility to ALL in adult patients.

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Conflict of Interest: None

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