Zainab Mohammed Jasim et al /J. Pharm. Sci. & Res. Vol. 11(6), 2019, 2285-2287

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Detection the Genetic polymorphism with risk incidence of blood leukemia patients in Hilla/Babylon

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Abstract:

Human Multi drug resistance (MDR1) gene was paly vital role in chemotherapy, transporting exogenous and endogenous materials from the cells.MDR1 gene can increasing genetic variation .Our results were performed to estimate the gene polymorphism at site C1236T to advancement and improvement of leukemia patients. In this research, specimens was consisted 70 acute leukemia cases, and 40 healthy samples for the detected of Human Multi drug resistance C1236T polymorphism , using the PCR-RFLP techniques. Acute leukemia patients had a significantly higher frequency of the C1236T genotype compared with the control group. In the present study, the results were showed that the *MDR1* C1236T genotype can affected risk of development of acute leukemia.

INTRODUCTION:

Acute leukemia is generally considered as a life threatening stem cell disease which is characterized by found of myeloid blasts in the bone marrow. It is may be estimate that there are 352,000 new status individuals with leukemia, and 265,000 people died from leukemia in 2012 worldwide [1] . There are two main types of acute leukemia, acute lymphoblastic leukemia and acute myeloid leukemia. Some early studies have showed that many harmful factors play significant role in the advancement of this disease, including tobacco smoking, down syndrome, long-term exposing to benzene, family history of cancers and ionizing radiation[2]. Genetic factors were play critical role in the pathogenesis of acute myeloid leukemia except environmental factors. Some of studies were recommended that a lot of factors were correleted with the sensitivity of leukemia and molecular factors[3].

Multi drug Resistance MDR1 is the best characterized as pump efflux transporters that is located on chromosome 21[4].

MDR1 gene was encoded a 170-kDa membrane transport protein called P-glycoprotein. For decrease the exposure of toxic materials to the cellular homeostasis, P-glycoprotein was expressed primarily in regions that play as epithelial barriers or act as secretory functions, including gastrointestinal tract, liver, kidney and blood-tissue barrier. Therefore, P-glycoprotein might play the role of sweeper by extruding several exogenous and endogenous materials, using ATP-dependent efflux pump[5].

[6] (2007) reported 50 single nucleotide polymorphisms in MDR1 gene associated with diseases related to pesticide exposure . MDR1 gene are responsible for inter individual variability in pharmacokinetics and works of many drugs. [7]was showed that over expression of P-gp was connected with deficiency proliferation activity and slower migration of enterocytes, that lead to prolonged resistance to apoptosis resulting in an increased chance of cell transformation. P-gp was play vital role in defending cells from risk materials and active metabolites; therefore, it can be admissible that this expression can share participate significantly in cellular response to stress stimuli. Promoter region of *MDR1* gene , was attached sites for various transcription factors. These sign clearly point out the possibilities of *MDR1* gene arrangement by a numerous of environmental signals. Further sign was reported that protein kinase C activation which increases P-gp efficacy also showed positive influence on gene expression of MDR1[8].

In this study, we showed association between polymorphisms in the MDR1 gene of C1236 T and risk leukemia.

METHODS:

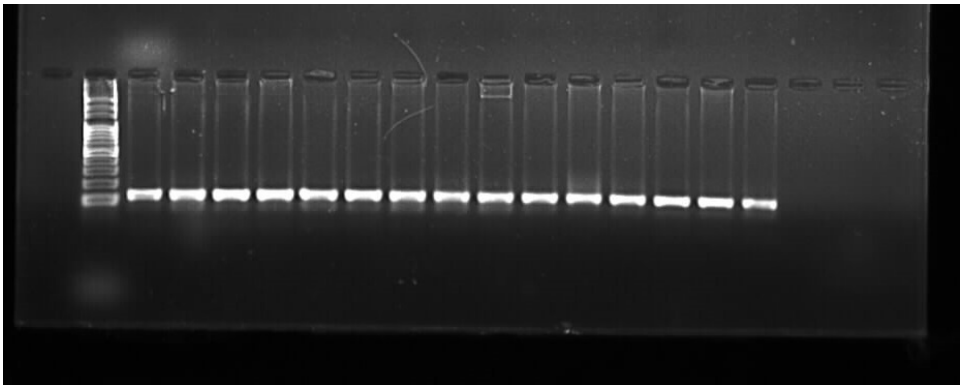
70 acute leukemia cases and 40 control samples were used in this study. Blood specimen was extracted from diagnostic patients that carry acute leukemia which obtained from Merjans hospital. The age and sex control samples were included (19-61 years) , male and female selected from different areas of hospital. Genomic DNA was isolated by extraction by special purification kit (Faverogen) due to the manufactures protocol.

Genotyping methods

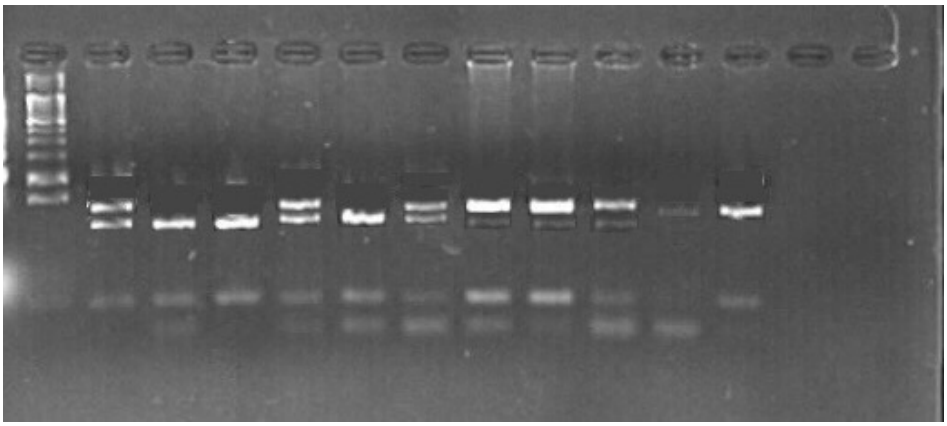
Specimens of all patients and controls that carry *MDR1* C1236T polymorphism were identified by PCR-RFLP methods. The C1236T polymorphism of *MDR1* was performed after PCR amplification using forward primer 5' TGT GTC TGT GAA TTG CCT TGA 3' and reverse primer 5' ATC TCA CCA TCC CCT CTG TG 3' . PCR was carried out in 50µL of reaction mixture that contain PCR buffer (10mM Tris-HCl, pH 9.0 50mM KCl, 1.5mM MgCl2), 200µM of each dNTP, 1 unit of Taq DNA polymerase (due to Biotech, USA), 20µM of each primer and 100ng of genomic DNA. PCR amplification consisted of primary denaturation at 94C for 60s, annealing at 56C for 60s, extension at 72C for 60s, and final extension 72C for 5min. DNA fragments were digested with restriction enzymes separated by agarose gel electrophoresis 3.5%, and staining by Ethidium bromide that used for visualized.

Statistical Analysis:

Genetic analysis was performed using Chi-square($\chi 2$) test. These tests were significant correlated when p value was less than 0.05.



PCR product of *MDR1* gene C1236T 180 bp



Figure(2): PCR-RFLP product(180bp); wild type homozygote(CC), demonstrating 93 and 87bp fragments, polymorphic homozygote (cc) 87, 58, and 35 bp fragments, and Cc heterozygote 93, 87, 58, and 35 bp

Table 1: Allele frequency for MDR1 C1236T in acute leukemia patients and controls.

Genotype MBL2	Control	Patient	*P* value
CC	23	20	**0.01**
Cc	10	15	
cc	7	15	
Total number	40	50	
Allele frequency			
Allele	**Control**		**Patient**
C	0.7		0.73
c	0.3		0.27

RESULTS AND DISCUSSION

Genotyping of MDR1 C1236T polymorphism of ALL patients and controls was performed by PCR-RFLP assay, and the PCR product was 180 bp as shown in figure(1):

The digested PCR product(180bp) figure(2) showed 3 different patterns; wild type homozygote(CC), demonstrating 93 and 87bp fragments, polymorphic homozygote (cc) showing 87, 58, and 35 bp fragments, and Cc heterozygote, showing 93, 87, 58, and 35 bp fragments.

The allele frequency distribution of *MDR1* gene C1236T polymorphoism was showed in table 1

In this reserch, the frequency of C1236T genotype was found to be increased slightly in leukemia patient 0.73 compared to controls 0.7.

There was statistically significant between acute leukemia patients and controls group using Chi squre test. The role of C1236T genetic polymorphisms in the development of acute leukemia was investigated, and other finding indicated that the TT genotype of C3435T gene affected the susceptibility to acute leukemia when compared to CC genotype.

The P-gp was encoded by the MDR1 gene is an ATP dependent efflux pump transports inflammatory material and toxins from the intracellular to extracellular region.

Genotyping of MDR1 gene have been identified to change both the expression plane and work of P-gp[9].

Other studies were evaluating the impact of these specific MDR1 on P-glycoprotein work and mRNA expression that support the correlation. [10]was showed reduced P-glycoprotein actively connected with all three variants both individually and as haplotype when compared with the wild type and reported the influence of C1236T on P-glycoprotein functionally in vitro by using validated stable recombinant epithelial cells.

The present study was similar to Japanese and Serbians studies that reported C1236T group, genotype C1236T was more common than other genotype in our study, more than on hundred SNP have been recognized in the human MDR1 gene[11]. MDR1 gene is a part of the ABC family that encodes Pg-p an ATP dependent efflux pump which give protection to the cell to get rid of toxins and exogenous materials[12]. The CC genotype carrier exhibits a different prognosis while carries of the TT genotype were given higher risk of developing acute lymphoblastic leukemia than other individuals[13]. Another study on patients with acute myeloid leukemia reported that TT genotype were correlated significantly with shorter relapse time and survival rates compared to heterozygotes [14].

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