International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.12, pp 634-639, 2016

Influence of CYP3A5 gene polymorphisms and risk of chronic myeloid leukemia

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Abstract : Associations between polymorphisms for genes encoding enzymes involved in biotransformation of xenobiotics and susceptibility to several cancers have been shown in several studies. The aim of the present study is to investigate the influence of cytochromes P450 (CYP450) 3A5*2 &6 gene polymorphisms in susceptibility to chronic myeloid leukaemia (CML).
Keywords: Leukemia, CML, CYP3A5*2, CYP3A5*6, polymorphisms, PCR.

Introduction:

Leukemia is defined as the accumulations of single, hematopoietic cells in the bloodstream or lymph[1, 2]. Generic cells (hematopoietic stem cells) mature into either myeloid or lymphoid ancestral cells from which all other specialized cells arise[3, 4].Chronic Myeloid leukemia is a hematopoietic stem cell disorder that causes uncontrolled cell proliferation of white blood cells [5, 6]. CML results from a reciprocal translocation between the chromosome 9 which contains Abelson Kinase domain and the chromosome 22 which contains Breakpoint Cluster Region t(9;22) [7, 8]. As a result of this translocation the regenerated 2 chromosomes are called Philedelphia Chromosome (Ph). Approximately 95% of CML patients have this Ph chromosome. This Ph chromosome encodes for an oncoprotein called BCR/ABL, This oncoprotein has a tyrosine kinase activity which phosohorylates a number of substrates and causes increased mitogenic activation, uncontrolled cell proliferation and decreased apoptosis [8, 9].

The CYP3A enzymes are the most abundant form of CYP enzymes in the liver and consist of the four isoforms CYP3A4, CYP3A5, CYP3A7, and CYP3A43.CYP3A4 and CYP3A5 account for the majority of CYP3A activity in adults and metabolize a wide range of drugs. However, CYP3A5 is frequently inactivated in Caucasian populations due to the non-functional CYP3A5*3 genotype [10,11]. The CYP3A5 gene is a part of a cluster of cytochrome P450 genes: CYP3A4, 3A5, 3A7 and 3A43, localized on chromosome 7q21.1 position [12].

Experimental Part

Study Subjects:

The practical side of the study was done in the period between "September 2015 until June 2016".One hundred and fifty samples were collected. Two enrolled groups of subjects were involved in this study.

Patients:

This study includes 90 patients with Chronic Myeloid Leukemia (CML) admitted to Marjan Hospital. Patients were included (38 males and 52 females), with an age range (8-76) years, they were diagnosed by specialist physicians and selected in the current study. Blood and serum samples were taken from every patient and control having thoroughly examined.

3.1.2. Healthy control group

Sixty of actual healthy persons from various Iraqi populations were arbitrarily involved in the study.

Blood Sampling:

About five milliliters of venous blood were collected from each patient in the study. The blood was divided into two parts: one part (about two milliliters) was collected into EDTA containing tubes for genetic part. The second part of the blood was placed in gel tube for thirty minutes, then transferred to plain tube and serum was obtained by centrifugation at 3000 rpm for 15 min ; after that the serum collected and kept in the freezer (-20 ºC) until it was used for the immune and viral assay.

Isolation of genomic DNA:

Genomic DNA was used for molecular study by sequestered from the fresh blood, which collected in tubes of anticoagulant EDTA and for frozen blood samples we recommended using protease K were applied using for DNA purification; Promega Wizard genomic kits. The isolation of DNA depended on the 5 stage procedure utilizing salting out techniques [13, 14]:

- Lysis of the RBCs in the Cell Lysis Solution.
- Lysis of the WBCs and their nuclei in the Nuclei Lysis Solution.
- The cellular proteins were then removed by a salt out precipitation step using the Protein Precipitation Solution.
- The genomic DNA was concentrated and desalted by Isopropanol precipitation.
- The genomic DNA was rehydrated using the DNA Rehydration Solution.

The Protocol for DNA Separation:

Procedure which provided with Promega kit was recommend for DNA separation as reveled in bellow.

The Estimation of DNA Concentration and Purity:

The DNA concentration of samples was estimated by using the Nano drop by putting 2.5µl of the extracted DNA in the machine to detect concentration in ng/µL and the purity detected by noticing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9.

PCR Technique:

In this study conventional PCR was used to detect mutation genes by using two primers as shown in the following table. The primers were supplied by Bioneer (Korea) Organization as a lyophilized result of various picomols fixations. Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of 100 pmol/µl and kept as a stock in -20ºC, to prepare 10µM concentration as work primer re suspended 10 pmol/µl in 90 µl of free DNase/RNase to reach a final concentration 10µM.

Table (1) Sequence of primers

Genes	Primer		Size Product	References
CYP3A5* 2	Forward	5'- CTCTTTAAAGAGCTCTTT TGTCTCTCA- 3'	108	Sailaja1 *et al*., 2010
	Reverse	5'- GTTGTACGCCACACAGCACC - 3'		
CYP3A5* 6	Forward	5'- GATAGTTCTGAAAGTCTGTGGC - 3'	495	Sailaja1 *et al*., 2010
	Reverse	5'- GAGAGAAATAATGGATCTAAGAAACC -3'		

Detection of CYP3A5*2 and CYP3A5*6 genes

The CYP3A5*2 and CYP3A5*6 genes were amplification by conventional PCR using an alteration of the process designated by Sailaja*et al*., (2010). The primer sets manufactured by Bioneer, KoreaGradient condition of CYP3A5*2 is explained in the following table. The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix, 5µl forward and 5µl reverse primer in 20 µl of total reaction volume.

Table 2 gradient condition for CYP3A5*2

Initial denaturation	95	5	1
Denaturation	95	1	30
Annealing Zones	51-53-55-57-59-61	1	30
Extension	72	1	30
Final extension	72	7	1
Storage	4	∞	

Optimum result revealed with the temperature 53°C. Then PCR amplification was done as illustrated in the following table, to get PCR 155bp product for CYP3A5*2.

Table 3 PCR condition for CYP3A5*2

Initial denaturation	95	5	1
Denaturation	95	1	30
Annealing	53	1	30
Extension	72	1	30
Final extension	72	7	1
Storage	4	∞	

Electrophoresis was done on 2% Agarose gel using 1X TBE buffer system at 70V for 30 min and 100V for 30 min., then they were analyzed in a gel documentation system.While the gradient condition of CYP3A5*6 differs slightly from condition of CYP3A5*2, as explained in the following table. The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix, 5µl forward and 5µl reverse primer in 20 µl of total reaction volume.

Table 4 gradient condition for CYP3A5*6

Initial denaturation	95	5	1
Denaturation	95	1	30
Annealing Zones	49-51-53-55-57-59	1	30
Extension	72	1	30
Final extension	72	7	1
Storage	4	∞	

The clearest band was in 53C°.PCR amplification was done as mentioned in (3-16) table, to get 268 bp. PCR product for CYP3A5*6 [13, 14].

Statistical analysis

Eventuality information was statistically evaluates by achieved with Statistical Package for the Social Science (SPSS) 15.0. Chi square test was calculated to test the significance of differences in proportions and Genotype relationship with the existence of CML in addition to prognosis. P values calculated using two-tailed with significance level $p<0.05$.

Results & Discussion

CYP3A5*2 and CYP3A5*6 genetic polymorphism Association CML patients

In the present study, CYP3A5*6 was seen in both of the CML patients and controls with non-basic complexities between them, 65% in controland 61.1 % in patient so that CYP3A5*6 aren't valuable atomic mechanical gathering to perceive the tribulation.

polymorphism of CYP3A5*2 with decreased mRNA expression prompts medication toxic quality and coming about DNA hurt which might be accountable for contamination development. In the present study, basic decreased of CYP3A5*2 genotype (32.2%)in CML social affair was watched which displayed that the defeat the CYP3A5 expression related with specific allele which will be mutant might be cause for the storing up of xenobiotics or endogenous steroids in different tissues which may impel genotoxicity that provide the peril for susceptibility frailty.

Regardless of what might be ordinary, past studies reported essentially indistinguishable rates of CYP3A5*2 allele in both the leukemia gathering and controls [15, 16, 17, 18].

However past studies on strong tumors depicted a principal relationship of polymorphism of CYP3A5*2 with esophageal [19] non-little cell lung [20] and prostate advancements [21, 22, 23]. Petrova*et al* depicted that CYP3A5 assortments were not associated with the event of colorectal improvement in Bulgarian masses [24, 25].

This study recommends that nature of polymorphism for CYP3A5*2 in CML patients may be associated in giving broadened peril for CML change and contamination improvement. In this manner the examination of CYP3A5*2 quality polymorphisms may be helpful in keeping up the CML patients for improvement[26, 27, 28].

Table (5) show the relation between genes and sex

	Male		**Female**				**Male**		**Female**				
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	
CYP3A5*2	2	1.3	11	7.1	13	8.3	19	3.8	10	2.0	29	5.7	<0.005
CYP3A5*6	16	10.3	23	14.7	39	25.0	24	4.7	31	6.1	55	10.9	

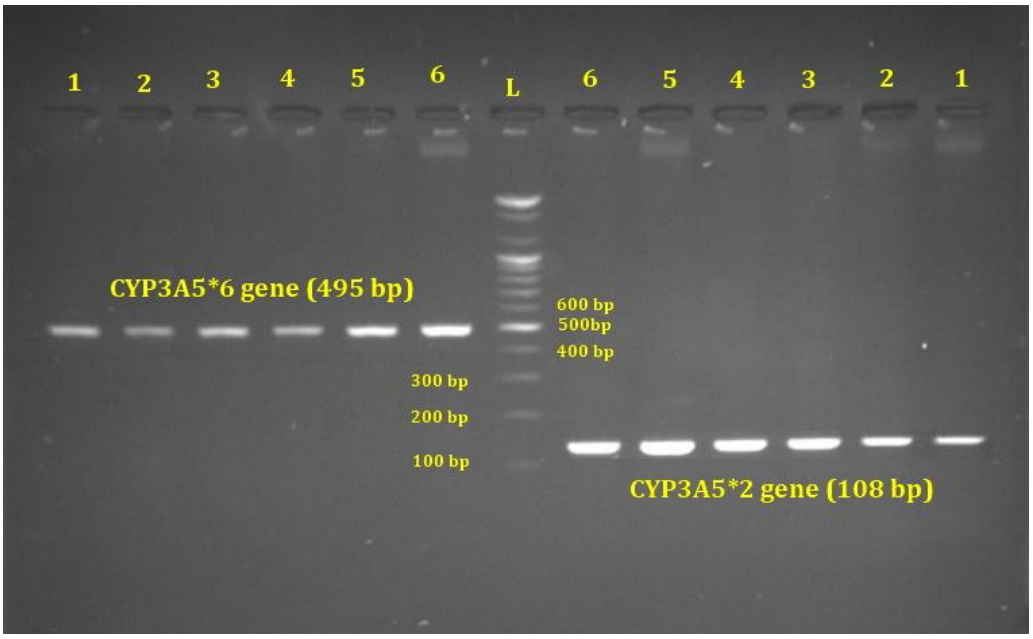

Figure (1) electrophoresis of PCR products for CYP3A5*6 and CYP3A5*2 gene. From left; lanes 1-6 revealed the CYP3A5*6 (495 bp) From right; lanes 1-6 revealed the CYP3A5*2 (108 bp) L lane contain the 100bp DNA Ladder, 5 % NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.5µl ethidium bromide

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