International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 166-173, 2016

Incidences of glutathione-Stransferase genotypes amongIraqi patients associated with chronic myeloid leukemia

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Abstract : Chronic myeloid leukemia (CML), as the vast majority of tumors results from a mind boggling communication between hereditary or non-hereditary variables. Exposures to xenobiotics endogenous or exogenous connected with a lessened individual capacity in detoxifying action, constitutes a danger of creating tumor. It is realized that polymorphism of glutathione S-transferases (GSTs) qualities influences the detoxification of xenobiotics. In this manner, we directed a case-control study in which 90 patients. Quality polymorphisms are viewed as a danger component for leukemia in various populaces. In this work we examined the GSTT1 and GSTM1 polymorphisms utilizing multiplex PCR for patients with endless myeloid leukemia (CML) contrasted and control bunch.
Keywords: Leukemia, CML, GSTT1, GSTM1, polymorphisms, PCR.

Introduction:

Leukemia by and large populace results from complex association between numerous hereditary and natural elements after some time. The transaction of xenobiotic introduction, endogenous physiology, and hereditary variability of numerous loci may encourage learning about leukemia etiology and the distinguishing proof of people who are at expanded danger of creating leukemia [1].

Perpetual myeloid leukemia (CML) is a blood tumor auxiliary to the nearness of the complementary translocation t(9; 22)(q34; q11) or BCR-ABL quality combination bringing about a harmful expansion of hematopoietic cells [2]. Be that as it may, the etiology of CML like the majority of tumors, results from an intricate collaboration between a few variables, among others presentation to ionizing and non-ionizing radiation, cancer-causing agents present in the earth, for example, benzene, smoke and pesticides [3 and 4].

The GST gathering is known not coded for by 16 qualities in six GST subfamilies, known as alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT) and zeta (GSTZ). Two across the board hereditary polymorphisms that include cancellations in GSTT1 and GSTM1 have been accounted for to prompt loss of catalyst movement [5] and have been explored in a wide range of populaces, including those from Japan [6], Italy [7] and Spain [8]. Besides, a few studies have recommended that vulnerability to intense and perpetual myeloid leukemia (AML and CML separately) could be identified with GSTT1 and/or GSTM1 erasures [9, 10].

Shifted results have been accounted for on the GSTM1 and GSTT1 invalid genotype and its relationship to CML in various ethnic populaces [11, 12]. Till date no study has been done to comprehend the etiology of CML in Kashmir. The principle point of the present study was to examine the impact of the polymorphism of GSTM1 and GSTT1 qualities on the defenselessness of CML in Kashmiri populace.

Both human GST cancellations, albeit to a great extent offset covering substrate affinities inside the GST superfamily, have outcomes when the living being comes into contact with unmistakable man-made chemicals. This seems important in modern toxicology and in medication digestion system [13, 14]. Plenty of studies from different nations on various geographic and racial gatherings have demonstrated changed results on the rate of genotypic variety of invalid genotype recurrence of GSTM1 and GSTT1 qualities in control populace and in addition patients with interminable myeloid leukemia [15, 16]. In this manner inferable from the significance of GST invalid genotype as danger component in different tumors and the relative contrasts in the recurrence of its event in different ethnic populaces, the present study was gone for giving important confirmation based information from North Indian populace to the learning of GSTM1 and GSTT1 polymorphism and further to assess the part of GSTM1 and GSTT1 invalid genotypes in the defenselessness to create CML.

Be that as it may, in spite of the fact that movement through all stages is most basic, the time course for movement can be to a great degree changed, and 20% to 25% of patients advance straightforwardly from CP to BP [17]. CML constitutes around 15-20% of all recently analyzed instances of leukemia in grown-ups and happens with a frequency of roughly 1-2 cases in 100,000/year, with an expected survival rate of 90% at 5 years and a yearly death rate of 2% [18, 19]. The middle time of onset of CML is around 40-60 years, with under 10% of the cases in patients matured under 20 years [20, 21 and 22].

Patients and Methods

Study Subjects:

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

Patients and Laboratory criteria for diagnosis

This study includes 90 patients with Chronic Myeloid Leukemia (CML) admitted to Marjan Hospital. Patients included (51 males and 63 females), with an age range (8-76) years, they were diagnosed by specialist physicians and selected in the current study. Blood and serum samples taken from every patient and control having thoroughly examined. The complete Blood Count (C.B.C.) and Fluorescent In Situ Hybridization (FISH) was done

Healthy control group

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

3.3.1. 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. 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 immunoassay.

3.3.2. Isolation of genomic DNA:

Genomic DNA was used for molecular study by sequestered from the fresh blood which collected 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 (Sambrook et al., 1989):

- 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 four types of PCR were used include ARMS, RFLP, multiplex and conventional PCR, to detect mutation genes by using ten 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

GENE	Primer		bp.
GSTT1	Forward	5'TTCCTTACTGGTCCTCACATCTC-3'	480
	Reverse	5'-TCACCGGAT CATGGCCAGCA-3'	
GSTM1	Forward	5'GAACTCCCTGAAAAGCTAAAGC-3'	219
	Reverse	5'-GTTGGGCTCAAA TATACGGTGG-3'	

PCR protocol

A successful PCR program depends on the reaction conditions including reagents, temperature and the prevention of contamination. Previous study indicates that PCR is sensitive to reaction condition and that the optimization of these conditions is necessary to reach the highest specificity and product yield. Standard amplification conditions were applied in PCR with primer sequences.The annealing temperature in which primers hybridize to complementary sequences on the template DNA is perhaps the most critical in PCR programming. The annealing temperature for PCR primers is based on melting temperature (Tm) calculations. Tm is the temperature at which half of DNA strands are denatured. A Tm calculation for PCR primers is based on guanine and cytosine (G+C) content. The annealing temperature is usually below the Tm in 2-12 °C. The primers concentration represents the optimal concentration. A decrease in the primer concentration leads to weak PCR product while an increase could result in the formation of primer dimer artifact, leading to misinterpretation of results (23, 24, 25 and 26).

The PCR based techniques do not require highly purified DNA preparations as it works well with partially purified DNA samples. However, DNA extraction may contain inhibitory compounds, like detergents used in cell lysis and protein denaturation in addition to other inhibitory compounds that could interfere with PCR leading to a reaction failure.

Detection of GSTT1 and GSTM1

The GSTT1 and GSTM1 were explored through conventional PCR technique using the process described by Kassogue and et al., 2015 with modification on it. The primer sets manufactured by Bioneer, Korea. Sequences of GSTT1 primer were:

5– TTCCTTACTGGTCCTCACATCTC– 3;

5–TCACCGGATCATGGCCAGCA–3

The primer sequences of GSTM1 were:

5'-GAACTCCCTGAAAAGCTAAAGC-3'

5'- GTTGG GCTCAAATATACGGTGG-3'

PCR optimization was done as a first step by using a gradient temperature. This is highly important to determine the optimum annealing temperature. The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix, 5µl of each forward and reverse primer in 20 µl of total reaction volume.

Table (2) gradient condition for GSTT1

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Annealing Zones	55-57-59-61-63-65	1	35
Extension	72	1	35
Final extension	72	7	1
Storage	4	∞	

Table (3) gradient condition for GSTM1

Step	Temperature C°	Time/min.	Cycles
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	∞	

After resolve of optimum annealing temperature for GSTT1 and GSTM1 genes by selecting the clearest band which is 63 C° for GSTT1 and 57 C° for GSTM1, PCR mixture was 5µl DNA,5µl master mix, 1.5 forward and reverse primer.

PCR condition for GSTT1 and GSTM1 were performed as in the following tables. Results of PCR amplicons were broke down on two percent of agarose gel recolored with 0.5 µg/ml ETBR. The GSTT1 and GSTM1 produce 480bp and 219bp separately (Kassogue et al., 2015).

Table (4) PCR condition for GSTT1

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Annealing	63	1	35
Extension	72	1	35
Final extension	72	7	1
Storage	4	∞	

Table (5) PCR condition for GSTM1

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	95	5	1
Denaturation	95	1	30
Annealing	57	1	30
Extension	72	1	30
Final extension	72	7	1
Storage	4	∞	

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

Glutathione S-transferase (GSTT1 and GSTM1) gene polymorphism correlated to CML patients

Glutathione-s-transferases (GSTs) are a family of multifunctional detoxifying enzymes that stimulate the junction of glutathione with great number of anticancer, chemical compound, ecological contaminants and agents (27, 28). Glutathione-S-transferases are closely linked with cancer as they are increased in blood and tissues of cancer patients. GSTs have been known as a tumor marker (29, 30).

GST families can be divided to three mammalian, microsomal GST cytosolic and mitochondrial. Cytosolic GST isoenzymes are comprehensively cytoprotective, though microsomal proteins have genius incendiary exercises. Cytosolic human GST show hereditary polymorphisms and this refinement can expand powerlessness to carcinogenesis and incendiary maladies (28). Knowledge of variety in recurrence of GSTT1 and GSTM1 invalid genotypes in various populaces may disclose differential reactions to poisonous chemicals (31). Currently, the effeteness of GSTM1 and GSTT1 polymorphism in tumorigenesis, for instance have been shown in various epidemiological studies (32, 33).

One of aims of this study was to define GSTT1 & GSTM1 genotypes in cases of chronic myeloid leukemia through conventional PCR technique and correlate this expression with the laboratory data and clinical outcome. In the current study, we noticed a significant difference of the GSTM1 present genotype in patients (**44.1**%) and in control (**25.5**%), table 6.

Table (6) show the correlation between gene polymorphism to CML and control

Gene polymorphism	Control 60		CML 90		P value
	No	%	No	%	
GSTM1	**11**	**25.5**	**49**	**44.1**	<0.003
GSTT1	**32**	**74.5**	**62**	**55.8**	

Shockingly, in considering members as per sexual orientation, we have discovered relationship between GSTT1 present genotype and CML in male (69.6%) but not in female as showed in Table 7.

This outcome may be cleared up by separations in xenobiotics presentation amongst both genders (e.g. smoking, Alcohol... etc) and other inherited features to the host. Something else, a study conveyed a relationship between invalid CML patient with GSTT1 and genotype which revealed that there is significant

difference (26). A different research arranged in Asia, moreover build up that GSTT1 invalid genotype was connected with the change of CML (33).

Table (7) show the relation between genes and sex

Genes	Gender/Control 60				Total		Gender/CML 90				Total	
	Male		Female				Male		Female			
	No	%	No	%	No	%	No	%	No	%	No	%
GSTM1	8	18.6	3	7.0	11	25.6	17	30.4	32	58.2	49	44.1
GSTT1	18	41.9	14	32.6	32	74.4	39	69.6	23	41.8	62	55.9

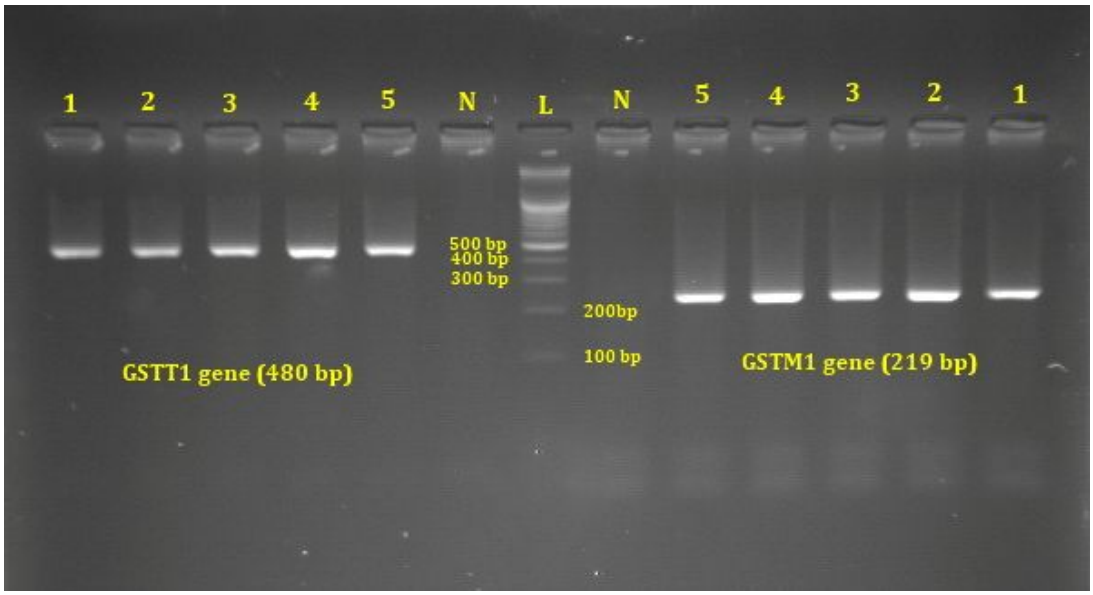

Figure (1) electrophoresis of RFLP-PCR products for GSTT1 and GSTTM1 gene. From left; lanes 1-5 revealed the GSTT1 (480 bp) From right; lanes 1-5 revealed the GSTM1 (219 bp) L lane contain the 25 bp DNA Ladder, 5 % NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.5µl ethidium bromide

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