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Frequency Of GSTP1 (Ile105Val) Gene Polymorphism In Iraqi CML Patients And Its Association With Susceptibility To CML

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Abstract:

Glutathione S transferase pi class (GSTPs), are dimeric phase II enzymes, and one of the most important cellular detoxification systems. They play several biological roles, including protection of cellular DNA against oxidative damage that can promote carcinogenesis; hence, their activity may alter cancer risk. A growing body of evidence has revealed a potential relevance between functional polymorphisms within gene encoding for GSTs л, and susceptibility to chronic myeloid leukemia. The lack of conclusive data has prompted our research aimed at evaluating the frequency of Glutathione S transferase P1 (Ile105Val) gene polymorphism in Iraqi chronic myeloid leukaemia patients and its association with susceptibility to CML. We conducted a case-control study on 40 adult ph-positive CML patients, in addition to 40 apparently healthy sex and age matched individuals serving as control group. 'PCR-Restriction fragment length polymorphism' technique was emplemented to detect (Ile105Val) polymorphism. Our results revealed more than threefold higher CML risk among subjects exhibiting the variant genotype (P=0.014; OR= 3.095). Thus indicating that (Ile-105-Val) polymorphic variants of GSTP1 gene might confer a higher risk of CML development.

Key words: GSTP1 (Ile105Val), Chronic Myeloid Leukemia, Single Nucleotide Polymorphisms**,** Susceptibility.

INTRODUCTION

Chronic myeloid leukemia (CML) is a type of haematologic malignancy characterized by uncontrolled expansion of neoplastic myeloid cells in bone marrow. It is depicted by the characteristic rearrangement of the long arms of chromosome 9 and 22 [t(9;22)(q34;q11)] ensuing a derivative 9q+ and a shortened 22q-, generating the so called "Philadelphia chromosome"(1). Ph chromosome harbors the fusion oncogene (BCR-ABL), which codes for the chimeric oncoprotein BCR-ABL1; a tyrosine kinase (TK) activating altered signaling pathways. The resulting phenotype is characterized by an unrestrained proliferation, inhibition of apoptotic signals and expansion of progenitor population, resulting in the manifestation of CML (1, 2, 3). CML is one of the most common types of leukemia accounting for 13.7% of newly diagnosed adults and nearly 15% of all leukemia cases. It is frequently diagnosed in adults and rarely in children. The average age at diagnosis is about 65. The incidence rate adjusted to age is approximately 2 cases /100000 people, which increases proportionally with age every year (4, 5, 6). The disease has a tri-phasic course including: chronic phase (CML-CP); accelerated phase (CML-AP); and blast crisis phase (CML-BC. CML is most commonly diagnosed in the chronic phase and merely 10% of cases are diagnosed in advanced phases. In the accelerated phase, disease becomes aggressive and may eventually evolve into blast phase; a final more serious phase with dismal outcome and symptoms analogous to AML (7, 8).

Although the genetic mutation responsible for CML leukemogenesis (bcr-abl rearrangement) has been identified, it is unclear what triggers this mutation (9). Like the majority of cancers, CML is the culmination of intricate synergy between genetic and environmental factors. Accumulation of xenobiotics whether endogenous or exogenous in the body can be genotoxics (10, 11). Glutathione S transferases pi class GSTPs are dimeric enzymes and member of glutathione transferases (GSTs) superfamily that play several critical biological roles, including protection of cellular DNA against oxidative damage that can promote carcinogenesis by inducing DNA damage (12). There are single nucleotide polymorphisms (SNP) arising within genes encoding for glutathione S transferases, that are associated with a greater risk of cancer and may also be associated with the phenomenon of drug resistance(13, 14). GSTP1 gene polymorphism is mostly a point mutation (SNP) within exon 5 [Ile105 Val] that results in reduced enzymatic activity of the protein(15). Inter-individual variances in the ability to activate pro-carcinogens or detoxify prospective carcinogen may account for great differences in cancer susceptibility. Thus, a possible link between polymorphisms within genes encoding for xenobiotic-metabolizing enzymes and increased CML risk has been proposed (16). It has been claimed that GST gene polymorphisms (in particular, GSTP1-1 variants) may act as factors modulating the risk of developing cancer(13). Although several studies have scrutinized potential risk factors for CML, it remains under investigation which genetic variation may influence CML risk or responses to treatment.

To the extent of our knowledge, GSTP1 (Ile105Val) gene has never been studied in Iraqi CML patients, hence this study is a humble attempt toward a better understanding of the potential link between the heterogeneous genotypes of GSTP1 Ile105Val gene and cancer risk in Iraqi CML patients.

PATIENTS AND METHODS

A total of eighty participants were recruited in this case-control study including Forty Iraqi CML-CP patients referring to The National Center of Hematology /Mustansiriyah University in Baghdad city/Iraq, in addition

to 40 unrelated, apparently healthy sex and age matched individuals serving as control group in the period between November 2017 and July 2018. Medical records of recruited patients concerning disease phase, treatment received, clinical and diagnostic laboratory data were thoroughly reviewed. Patients were also subjected to detailed history taking, thorough clinical examination and laboratory investigations: including; complete blood count (CBC), liver and renal function tests, S. uric acid, lactate dehydrogenase test and coagulation screen.

DNA isolation and GSTP1 (codon 105) polymorphism genotype analysis

Three ml of peripheral blood was obtained in an EDTA tube and genomic DNA was extracted using Quick-gDNA™ Blood MiniPrep [Cat. No. D3072 & D3073; Zymo/USA] following the manufacturer protocol. The extracted genomic DNA was then preserved at <- 20°C until PCR was performed

GSTP1 (codon 105) polymorphism was analyzed using 'polymerase chain reaction-restriction fragment length polymorphism [PCR-RFLP] technique'. A PCR mixture was prepared using master mix tubes [Maxime PCR PreMix kit, Intron / Korea, Cat. No. 25025]. The mixture comprised of: 1.5µl of genomic DNA, 5µl of Taq PCR PreMix, 1µl of each of Primers (Forward 5'-GTA GTT TGC CCA AGG TCA AG - 3' and Reverse 5'-AGC CAC CTG AGG GGT AAG- 3'), in addition to 16.5µl of distilled water. Optimal PCR cycling protocol for analysis of GSTP gene was then set as: initial denaturation step for 3 minutes at 94°C, followed by 35 cycles of three steps: denaturation at 95°C for 15 seconds, annealing at 68°C for 35 seconds and extension at 72 °C for 90 seconds. The final extension step was performed at 72°C for 5 minutes. After PCR, amplification, five µl of PCR products were subjected to restriction digestion using 0.5 µl of restriction enzyme: BsmAI (Biolab/NewEngland) in 10µl final volume; at 37°C for 25 minutes, for genotyping of studied samples. Gel electrophoresis of GSTP1 RFLP product demonstrated four band at (329,216,113,107 bp) the representing heterozygous variant (AG); three band at (216,113,107 bp) representing the homozygous variant (GG); and two bands at (329, 107 bp) indicating the presence of homozygous wild type (AA) (see Figure 1).

Statistical analysis: Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. Independent t-test was used to compare the continuous variables among study groups accordingly. Categorical frequencies of numerical data were compared using Chi square test. The allele and genotype frequencies were calculated by direct counting. Multiple group variables were compared using analysis of variance (ANOVA) at a confidence interval of 95%. P-values of <0.05 were considered statistically significant.

RESULTS

In this case-control study, we successfully recruited 80 participants including: 40 ph-positive CML-CP patients with mean age of 53.65±13.8 ranging from 19 to 80 years (16 males and 24 females), in addition to 40 apparently healthy subjects with mean age of 54.18 ±13.58 ranging from 21 to 82 years (16 males and 24 females). The highest distribution of patients (62.5%) was in [40 – 64 yrs.] age group.

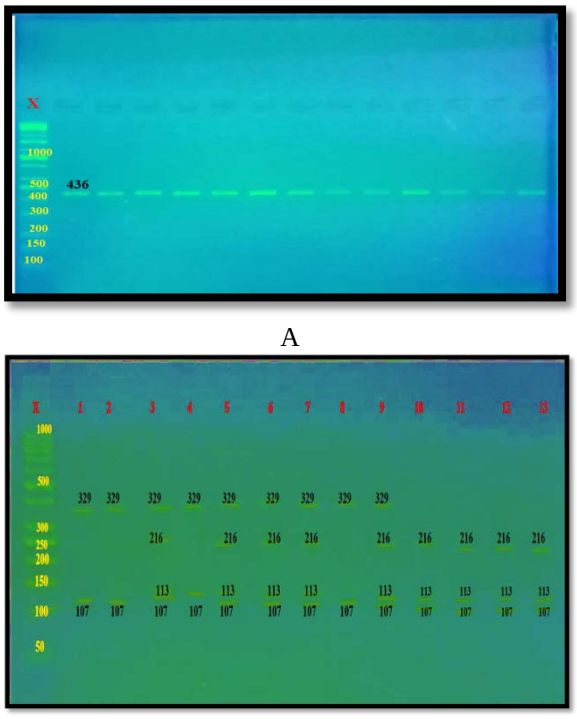

Figure 1: A- electrophoresis of PCR product showing 436bp band, X represents 100 bp ladder; B-gel electrophoresis of GSTP1 RFLP product. Lane: X represents 50 bp ladder. Lanes 1, 2 and 8 demonstrate homozygous wild type (AA); lanes 3,5,6,7 and 9 show heterozygous variant (AG) and lanes 10.11,12 and 13 show homozygous variant (GG).

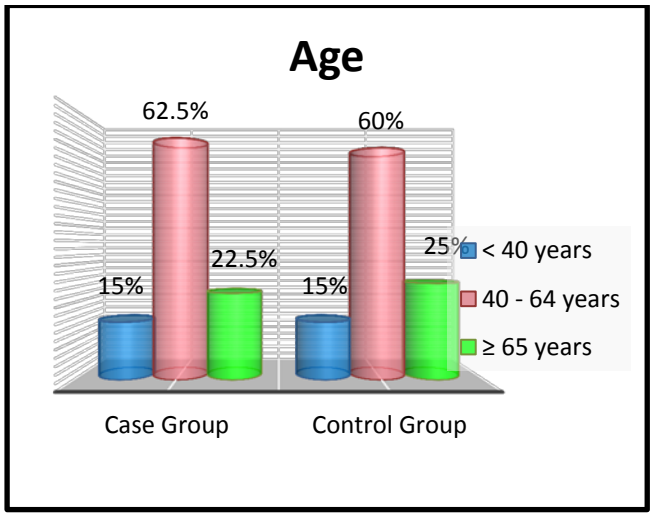

Figure 2: Distribution of study subjects' groups by age

The distributions of genotype frequencies and allele frequencies of the GSTP1 gene (Ile105Val) polymorphism studied among patient and control groups are summarized in **table -1.**

Table -1: Frequency distribution of GSTP1 gene polymorphism

Study Group	Genotype			Allele Frequency	
	Ile/Ile No. (%)	Ile/Val No. (%)	Val/Val No. (%)	Ile	Val
Case	15 (37.5)	21 (52.5)	4 (10.0)	0.64	0.36
Control	26 (65.0)	10 (25.0)	4 (10.0)	0.78	0.22

Table -2: Association between study groups and GSTP1 genotypes

Variable	Study group		Total (%) n=80	Odd's Ratio	95% C.I of odd's ratio	P- value
	Case (%) n= 40	Control (%) n= 40				
Genotype						
Wild	15 (36.6)	26 (63.4)	41 (51.2)	**3.095**	**1.24 – 7.7**	**0.014**
Variant	25 (64.1)	14 (35.9)	39 (48.8)			

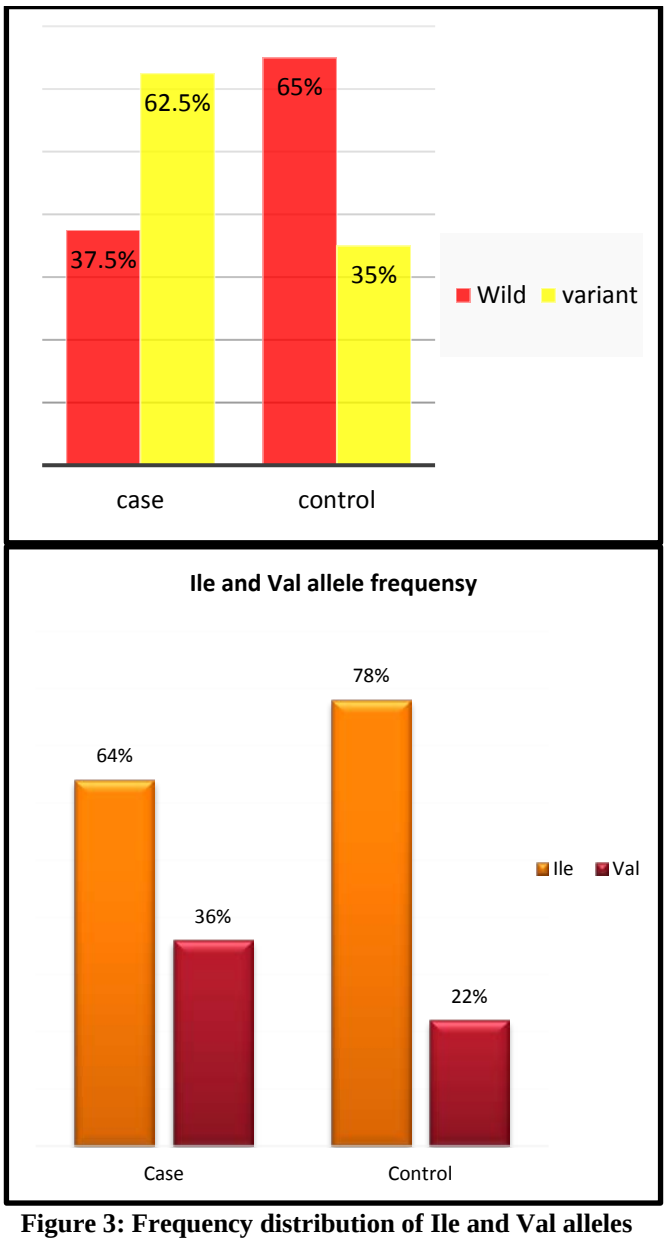

Figure 3: Frequency distribution of Ile and Val alleles of GSTP1 among patients and control

Our results showed that the heterozygous variant (IIe/Val) was more frequently represented among CML patients (52.5%) compared to (25%) in controls, while the homozygous wild type (IIe/IIe) was more frequently exhibited among controls with 65%. However, the homozygous variant Val/Val was equally distributed in both patients and controls [figure -3].

Upon comparing combined variant with wild GSTP1 polymorphic genotypes in case and control groups, the highest prevalence of the variant genotype (IIe/Val + Val/Val) was found in CML patients with a percentage of 64.1% with statistically significant association between CML and GSTP1 (Ile105Val) genotype. Calculated odds ratio revealed more than threefold increased risk of CML among subjects exhibiting the variant genotype (**P=0.014; OR= 3.095**) [see **table-2**]. Investigating the relation between variant GSTP1 genotypes and age at diagnosis, presentation, haematological parameters among CML patients failed to reveal a statistically significant difference with ($P > 0.05$).

DISCUSSION:

The π class of GST enzyme is a major enzyme class involved in the inactivation and detoxification of various mutagenes, carcinogens and other toxic chemicals (17). Polymorphic variation of GSTs have been linked to susceptibility to several benign and malignant disorders such as acute myeloid leukemia, gastrointestinal tumor, non-small cell lung cancer and prostate cancer (18, 19).

In this case–control study, we attempt to evaluate the potential effects of genetic polymorphisms of GSTP1 gene in a sample of Iraqi CML patient on chronic myeloid leukemia susceptibility

Our results revealed that the frequency of the combined variant genotypes (IIe/Val + Val/Val) was higher in CML patients 62.5% when compared to that of the controls, whereas the frequency of the wild genotype (Ile/Ile) was higher in controls at 65%. In addition, the heterozygous variant type (IIe/Val) was more frequently represented among CML patients at (52.5%), whereas 65% of controls exhibited the homozygous wild type (IIe/IIe).

Polymorphisms within genes encoding the GSTP enzyme, presumably, alter cancer risk by "differential ability to conjugate and detoxify both endogenously formed and exogenously derived electrophiles and their metabolites"(18). Thus, individuals who have diminished ability to detoxify toxic xenobiotics from the body are at a higher risk to develop cancer (20). Hence, an association has been proposed between the polymorphic forms of the enzymes involved in xenobiotics metabolism and the altered risk to several cancers including chronic myeloid leukemia.

As for allele frequency, Ile allele frequency was higher in controls, while Val allele frequency was higher among CML patients. This can be appertained to varied catalytic ability to metabolize chemical carcinogens and mutagens, and altered thermal stability of the encoded enzyme by the mutant genotype. As the enzyme encoded by the Isoleucine variant is more efficient in xenobiotics detoxification than the enzyme encoded by Valine allelic variant which is associated with less stability and higher level of DNA adducts eventually increasing the risk of neoplasia (18, 21, 22).

Our results also revealed a higher prevalence of the variant genotype (IIe/Val + Val/Val) in CML patients with a percentage of 64.1% and more than threefold increased risk of CML among subjects exhibiting the variant genotype with a statistically significant association between CML and GSTP1 (Ile105Val) genotype. (P=0.014; OR= 3.095) suggesting that the variant GSTP1genotype confer a potential susceptibility to CML. This is in accordance with results from other investigators (Sailaja and Hamed) who also reported a possible association between the GSTP1 (Ile105Val) polymorphism and CML tumorigenesis (23, 24).

Hamed reported that the frequency of GSTP1 variant heterozygous (Ile/Val) was higher in CML patients at (52.5%) when compared to controls (26.6%), while the frequency of GSTP1 wild genotype was higher in controls (70%) than in CML patients (37.5%). He also reported that the variant genotypes (IIe/Val + Val/Val) were more frequent among adult CML patients (62.5%) compared to (39.9%) in controls, with fourfold increased risk of CML (24). Claudia *et al.* described similar results in 186 CML cases from Romania, showing that the allele frequency of GSTP105Val was higher in CML patients (22.9%) than in controls (17.4%). They also suggested that the homozygous variant genotype might contribute to the risk of developing CML (20). On contrary, a study by Karkucah on 71 Turkish CML patients provided no evidence of a relationship between the GSTP1 IIe105Val polymorphism and susceptibility to CML (25).

An association between the polymorphic variants of the GSTP1 gene and the altered risk to other haematological cancers was also investigated in several studies. In a study by Dunna performed on 143 AML patients and 147 ALL patients, the homozygous mutant genotype Val/Val of the GSTP1 Ile105Val polymorphism was associated with susceptibility to acute leukemia and was also associated with inferior outcome (26). Bənescu also suggested that the presence of mutant GSTP1 genotype increases the risk of acute myeloid leukemia (27). Furthermore, a study by Delamain presented an evidence that GSTP1 polymorphism might serve as an independent prognostic marker in de novo diffuse large B-cell lymphoma and affects survival, as well as toxicity and response to chemoimmunotherapy (21). On the other hand, a study by Guven to evaluate the role of polymorphic GSTP1 Ile105Val genotypes on the risk of childhood ALL in the Turkish population, showed no association between GSTP1 variants and the susceptibility to childhood ALL (28). The lack of concordance between results could be rationalized by varied influences of the GSTP1 genotypes on genetic susceptibility to leukemia in different populations and ethnicities due to specific gene–gene interactions, exposure to different types of mutagens and environmental carcinogens (gene–environment interactions), or both. Some authors proposed that the presence of valine allele confers a higher risk to develop CML at early age. This can be attributed to the reduced ability of the mutant variant to detoxify toxic metabolites as UV-Ray-derived oxidative stress and other environmental carcinogens (29). In conclusion: Ile-105-Val polymorphic variants of glutathione S transferase P1 gene might confer a higher risk of CML development and treatment failure. Additional studies are warranted aimed at substantiating the possible role of these polymorphic variants in CML pathogenesis.

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