Association of GSTP1 Ile-105-Val Gene Polymorphism with Response to Treatment Among Iraqi Chronic Myeloid Leukaemia Patients

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Abstract:
Background: Pharmacogenomics is a relatively new study field that synergize pharmacology with genomics, analyzing the correlation between genetic variation and pharmacokinetics among patients. In the current study, we evaluated the potential effect of functional polymorphisms within gene encoding for Glutathione S transferase pi class (GSTPs) and treatment response among chronic myeloid leukaemia (CML) patients. GSTPs are multifunctional phase II biotransformation enzymes. Their main biologic role is to catalyze the conjugation to endogenous glutathione (GSH). In addition, they can alter drug potency in malignant cells. Polymorphic variants of these enzymes have been implicated in inter-patients' variability in drug response and outcome in CML patients.

Aim of study: Evaluating the association between GSTP1 Ile-105-Val gene polymorphism and response to treatment among Iraqi CML patients.

Method: A ‘PCR-RFLP’ assay was implemented to detect the polymorphic variants of codon 105 GSTP1 gene of forty Iraqi CML patients in chronic phase referring to the National Center of Haematology in the period between November 2017 and July 2018.

Results: Our result revealed a statistically significant association between the GSTP1 genotypes and response to treatment. The variant genotypes were associated with inferior log reduction in BCR-ABL1 mRNA and poorer responses comparing to the wild genotype with P-value of 0.006, and 0.034 respectively.

Key words: GSTPs, Chronic Myeloid Leukaemia, Polymorphism, Response.

علاقة تعدد الأشكال الوراثية للوراثة GSTPI Ile 105 Val مع الاستجابة للعلاج في مرضى سرطان الدم النقوي المزمن العراقيين

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الخلاصة:
ان تغاير الأشكال الوراثية للجينات المرتبطة بالتحولات الوراثية للادوية قد تكون مسؤولة عن الاختلافات بين الأفراد من حيث الاستجابة العلاجية، إذ أن تغايرات هذا التغييرات الجينية تؤثر على الاستجابة الدوائية للمرضى قد تساعد في اختيار العلاج المناسب والتثبيت النتائج للعلاج.

في هذه الدراسة تم إجراء التنميط الوراثي للمورث (جلوتاثيون أس ترانسفيريز بي 1) لرابعين مريضا بسرطان الدم النقوي المزمن العراقيين في المرحلة المزمن باستخدام تقنية "تغاير قسط التقني لتفاعل البلازما المبسط" ودراسة تباينات الانماط الجينية المختلفة على الاستجابة العلاجية للمورث.

ظهرت النتائج أن المرضى حاملن النمط الوراثي المتغير كانو أكثر عرضة للاستجابة ضعيفة للعلاج من المرضى حاملين النمط الوراثي. من هذا النتائج، ما زاد في خطر الاستجابة ضعيفة للعلاج لمرضى سرطان الدم النقوي المزمن، لذلك هناك حاجة لإجراء المزيد من الدراسات لإجراء تأكيد دور هذه التغييرات في حركة المواد الدوائية واستخدامها المحتمل كعوامل تنبؤ بالإستجابة العلاجية في المستقبل.

الكلمات المفتاحية: سرطان الدم النقوي المزمن، تغاير الأشكال الوراثية، جلوتاثيون أس ترانسفيريز بي 1.
Introduction:
Chronic myeloid leukemia 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 (Ph) 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]. Since the introduction of tyrosine kinase inhibitors (TKI), life expectancy of CML patients has dramatically improved, with 10-year survival reaching nearly 85% and 10-year relative survival exceeding 90% [4]. Thus, achieving survival rates comparable to that of the general population, with a recent study showing that patient survival may be determined by comorbidities rather than by the disease itself [5,6]. In the face of availability of novel potent TKIs, treatment failure remains a significant challenge in the treatment of CML [7] The potential causes leading to primary resistance and slow response have been explored in many studies. There were possible associations of therapy efficacy with expression levels of individual genes. Genetic polymorphisms were also investigated as possible prognostic markers [8]. The glutathione transferases (GSTs) are one of the most important cellular detoxification systems [9]. The GSTs superfamily is a group of ubiquitous multifunctional proteins, found in both eukaryotes and prokaryotes. In eukaryotes, GSTs are classified into three major families of proteins based on their cellular localization namely: mitochondrial, cytosolic and microsomal GSTs. Cytosolic GSTs are widely distributed and in turn sub-classified into eight main classes on the basis of their physical, structural and chemical properties [10]. They are: GST-alpha, GST-pi, GST-mu, GST-theta, GST-zeta, GST-kappa, GST- sigma and GST-omega [11]. The π-class of GSTs is the most abundant member of cytosolic glutathione transferases in the mammalian cells [12]. It accounts for approximately 90% of the GST family enzymatic activity, as it is widely expressed in many tissues. GSTP is encoded by a single gene, known as GSTP1[9,13]. Polymorphism of human GSTP1 is a common phenomenon and its variant alleles are frequently encountered [14]. Polymorphic GSTP1 harbors an A to G base substitution at codon 105 (exon5); nucleotide 313 that result in missense substitution of isoleucine by valine; Ile105Val (rs1695) [9,15]. Codon 105 residue constitutes part of the GSTP1 gene active binding site of hydrophobic electrophiles [16]. This substitution give rise to three GSTP1 genotypes, they are: Ile/Ile (isoleucine/isoleucine) homozygous wild type, Val/Val (valine/valine) homozygous and isoleucine/valine (Ile/Val) heterozygote variants [14]. Despite the lack of conclusive data, a growing body of evidence has revealed a potential relevance between polymorphisms within genes involved in binding, transport and metabolism of Imatinib and therapy outcomes among CML patients [17]. To the extent of our knowledge, this the first study addressing the possible link between functional polymorphisms within gene encoding for GSTs π and treatment outcome among Iraqi CML patients. 

Patients and Methods
Forty Iraqi CML patients referring to The National Center of Hematology at Mustansiriya University were recruited to participate in the study (including twenty-one optimal responders, twelve failed to respond and seven showing warning response). The study was conducted in Baghdad city/Iraq in the period between November 2017 and July 2018. The
patients were selected in accordance to the following inclusion criteria: 1- Adult philadelphia+ve CML patient, 2-In chronic phase [according to European Leukemia Net (ELN) definition of advanced phase CML disease], 3- undergoing treatment with (Imatinib) for at least 12 months [All patients received Imatinib (Glivec)® at a dose of 400 mg/day and debulking hydroxyurea]. Medical records of recruited patients concerning disease phase, treatment received, clinical and diagnostic laboratory data, were thoroughly reviewed. As regards response status, patients were classified into three categories: Optimal, Warning, and Failure (response to treatment was evaluated according to the current ELN guidelines).

Ethical consideration: This study was reviewed and approved by the Institutional Review Board (IRB) of the college of Medicine/ Mustansiriyah University. An informed consent was provided by all participants of the study after being well informed with the full objectives of the study and its prospective impact on individual's health. Data confidentiality is preserved in accordance to the revised (2008) Helsinki Declaration of Bioethics.

Blood collection and CBC: Five ml of venous blood was collected in an EDTA vaccutainer tube after permission was granted via verbal informed consent. A complete blood count was measured using 100µl of whole blood within one hour of blood collection by means of fully automated Hematology auto-analyzer (Convergy®X5 by Convergent Technologies/Germany).

BCR-ABL transcript level quantification: The level of BCR-ABL mRNA transcripts was measured using peripheral blood collected in EDTA tube that was either immediately processed or stored at 4°C for, using GeneXpert Diagnosis System (Version 4.6a by Cepheid/USA) BCR-ABL monitor kit according to the manufacturer’s recommendation. Results were expressed as percentage on the International Scale IS.

DNA isolation: A Quick-gDNA™ Blood MiniPrep kit was used to extract genomic DNA (following the manufacturer protocol). The extracted DNA was then preserved at -20°C until PCR was performed.

GSTP1 (codon 105) polymorphism genotype analysis: GSTP1 (codon 105) polymorphism was analyzed using ‘polymerase chain reaction-restriction fragment length polymorphism [PCR-RFLP] technique’. A 436 bp targeted gene was amplified implementing two sets of Primers (Forward: 5’-GTA GTT TGC CCA AGG TCA AG - 3’ and Reverse: 5’-AGC CAC CTG AGG GGT AAG- 3’.

PFLP assay was then performed on 5 µl of each of amplified PCR products using [BsmAI] restriction enzyme (Biolab/New-England). The products of RFLP analysis were then resolved on 2% agarose gel electrophoresis containing Red safe staining solution, then visualized using UV trans-illuminator. The size of the PCR-RFLP products was then assessed using a DNA molecular weight marker. Restriction digestion gave rise to: two bands at (329, 107 bp); three band at (216, 113, 107 bp); and four band at (329, 216, 113, 107 bp) indicating the presence of homozygous wild type (Ile/Ile), homozygous variant type (Val/Val), and heterozygous variant type (Ile/Val) respectively. Following the RFLP genotyping, 10 % of samples from each of the three different polymorphic genotypes were randomly selected and sent to the commercial company [Macrogen, Inc./Seoul, South Korea] for direct sequencing to confirm the results.

Statistical analysis: Data analysis was performed using Statistical Package for Social Sciences 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 current study, PCR-RFLP analysis was implemented for genotyping of 40 CML patients through BsmAI enzyme aptly digesting the targeted region on codon 105(exon5) of GSTP1 gene producing 3 distinct polymorphic genotypes: homozygous wild type Ile/Ile, homozygous mutant variant Val/Val, and heterozygous mutant variant Ile/Val [see Figure 1].
The mean age of the patients included in this study was: 53.65±13.8 (Mean±SD) years old ranging from 19 to 80 years. The highest proportion of study subjects was found in age group [40 – 64] years. The gender distribution was 16 (40%) male and 24 (60%) female [male to female ratio is 0.6:1]. Investigating the relation between variant GSTP1 genotypes and age at diagnosis among patients showed that the mean age at diagnosis for patients with variant genotype (Ile/Val + Val/Val) was 42.32±13.78 (mean±SD). Though it was lower than that of patients with wild genotype (Ile/Ile) 48±12.83 (mean±SD), it failed to reveal a statistically significant difference with p-value of 0.621 (P > 0.05), as shown in table 1.

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 and 11 show homozygous variant (GG).
Investigating the relation between the polymorphic GSTP1 genotypes and several haematological parameters also failed to reveal statistical significance (P > 0.05) as shown in Table 2.

Table 1: Difference in means of age at diagnosis between genotypes

<table>
<thead>
<tr>
<th>Age at diagnosis (Years)</th>
<th>Genotype</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Mean ± Std. Dev</td>
<td>variant Mean ± Std. Dev</td>
<td>0.621</td>
</tr>
<tr>
<td>48 ± 12.82</td>
<td>42.32 ± 13.78</td>
<td></td>
</tr>
</tbody>
</table>

With respect to response to treatment, our results revealed that the highest prevalence of treatment failure was observed in patients exhibiting the combined variant genotype [Ile/Val + Val/Val] at (48%) when compared to wild type. When comparing the individual variant genotypes to the wild type, both revealed a statistically significant association (P= 0.004) with half of patients with homozygous variant Val/Val failed to respond and other half showed warning response. As to heterozygous variant Ile/Val, 47.6% of patients carrying this genotype showed failure responses. On the other hand, 73.3% of patients carrying the wild type showed optimal responses [see table 3].

When investigating the association between log reduction in BCR-ABL1...
mRNA transcripts and GSTP1 genotypes, a statistically significant association was revealed with p-value=0.034. We noticed that 90% of patients with log of zero exhibited the homozygous variant genotype Val/Val. While the highest proportion of patients with log of 4 and 4.5 exhibited the wild type Ile/Ile (75% and 66.7% respectively) as shown in table 4, indicating that carriage of homozygous variant Val/Val might confer risk of inferior log disease reduction following IM therapy and higher residual leukemic burden.

**Table 3: Association between GSTP1 genotypes and response to treatment**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Response to management</th>
<th>Total (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimum (%)</td>
<td>Failure (%)</td>
<td>Warning (%)</td>
</tr>
<tr>
<td>Genotype (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Ile/Ile</td>
<td>11 (73.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Variant</td>
<td>Ile/Val + Val/Val</td>
<td>10 (40.0)</td>
<td>12 (48.0)</td>
</tr>
<tr>
<td>Genotype (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Ile/Ile</td>
<td>11 (73.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Heterozygous variant</td>
<td>Ile/Val</td>
<td>10 (47.6)</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>Genotype (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Ile/Ile</td>
<td>11 (73.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Homozygous variant</td>
<td>Val/Val</td>
<td>0 (0)</td>
<td>2 (50.0)</td>
</tr>
</tbody>
</table>

**Table 4: Association between PCR log and GSTP1 genotype**

<table>
<thead>
<tr>
<th>*Log reduction in BCR-ABL1 mRNA transcripts</th>
<th>Genotype</th>
<th>Total (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild (%)</td>
<td>Homozygous Variant (%)</td>
<td>Heterozygous Variant (%)</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Ile/Ile</td>
<td>Val/Val</td>
<td>Ile/Val</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>9 (90.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1 (50.0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>2</td>
<td>4 (57.1)</td>
<td>1 (14.3)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>3</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1 (100.0)</td>
<td>0</td>
</tr>
</tbody>
</table>

*1, 2, 3, 4, 4.5, 5 logs are equivalent to BCR–ABL1 transcript of ≤ 10%, 1%, 0.1%, 0.01%, 0.0032% and 0.001% IS respectively.
Discussion

Although the introduction of BCR-ABL targeted therapy (TKIs) has set the modern paradigm for the management of CML and have resulted in near-normal life expectancy, some patients develop resistance to TKI therapy, leading to therapeutic failure. Even with emergence of new TKIs with higher efficacy unfortunately, these novel TKIs are still hindered by drug resistance \[18,19\]. Nearly 10 to 20% of patients receiving TKI in the frontline setting faces treatment resistance with an additional 10 to 20% of patients failing therapy due to varying degrees of toxicity and intolerance. Nowadays, it is becoming clearer that mechanisms independent of BCR-ABL1 gene can contribute to TKIs resistance. Polymorphisms in genes encoding enzymes involved in drug uptake and metabolism have been implicated in inter-patients’ variability in drug response and outcome in CML patient \[20,15,21\]. Hence, we questioned whether the polymorphic GSTP1 genotype could be a predictor for response to Imatinib therapy. In current study, we noticed that the prevalence of treatment failure was significantly higher among patients carrying the combined variant genotype [Ile/Val + Val/Val] at (48%) when compared to non-variant wild type (P= 0.006) indicating a higher risk of treatment failure among patients exhibiting the variant genotype.

Regarding the association between response to treatment and individual genotypes (Val/Val) and (Ile/Val), both variant genotypes showed a statistically significant association with response to treatment when compared to non-variant wild type (P= 0.004) suggesting that carriers of either genotypes (Val/Val or Ile/Val) are more prone to achieve non-optimal responses following Imatinib therapy. Our results agree with a study by Makhtar et al. conducted on 278 Malaysian CML patients that reported a significantly higher frequency of heterozygous variant as well as homozygous variant of GSTP1 in Imatinib resistant group as compared to the Imatinib good response group. Makhtar proposed that carriage of heterozygous and homozygous variant GSTP1 genotypes can be associated with higher risk for development of Imatinib resistance \[17\]. This also concur with a study by Elhoseiny et al. in which the variant genotype was associated with poorer hematological response. Elhoseiny data showed that all patients who failed to achieve complete remission were harboring the mutant type [50% homozygous and 50% heterozygous] \[14\]. Furthermore, a study by Weich reported that GSTP1-Val/Val genotypes had short time to treatment failure in a group of TKIs unresponsive patients comparing to other GSTP1 genotypes. They also showed that patients carrying GSTP1-Val/Val genotype were significantly associated with reduced event free survival compared to those carrying other GSTP1 genotypes \[122\].

As it is common to describe the residual leukemic burden as a log10 reduction from the standardized baseline \[21\], next we asked whether the GSTP1 polymorphism had an impact on leukemic burden in terms of log reduction in BCR-ABL1 mRNA transcript. Our data unveiled a statistically significant association between log disease reduction and GSTP1 codon (105) polymorphic genotypes with most of patients achieving deep molecular response (i.e. > log 4 log reduction in BCR-ABL1 mRNA transcript from the standard base line) exhibited the wild type Ile/ABL. On the other end of spectrum, the majority of those who achieved inferior log reduction exhibited the mutant genotypes. Thus, we propose that GSTP1 codon (105) polymorphism may play a role in inter-patient's differences in residual leukemic burden following Imatinib therapy. Until now, there is no evidence-based explanation of precisely how GSTs modify the response to Imatinib treatment. Polymorphisms within GST are associated with a prominent substrate dependent structure–activity differences \[23\]. According to Traverso et al. the combination of elevated GSTs
expression along with high GSH levels, can escalate the rate of conjugation and detoxification of chemotherapeutic agents, thus decreasing their effectiveness. Moreover, beside their transferase function, GSTs have been shown to interact with members of the (MAP) kinases family including c-Jun N-terminal kinase 1 (JNK1) and apoptosis signal-regulating kinase 1 (ASK1), preventing their interactions with downstream targets. Since most anticancer drugs induce apoptosis via activation of those kinases, this novel, non-enzymatic role is directly related to the GST overexpressing phenotypes of many drug-resistant cancers [24]. Thus, owing to their multiple enzymatic and non-enzymatic functions, polymorphic variation within gene encoding the GSTP enzymes might explain inter-patient discrepancies in Imatinib pharmacokinetics, and might be fundam-ental determinants for response in Imatinib treated CML patients.

**In conclusion**, current study revealed that CML patients exhibiting either of the Valine 105 allelic variants of GSTP1 (heterozygous or homozygous) showed higher risk of treatment failure. Hence, polymorphic variation in GSTP1 gene might contribute to heterogeneous responses to Imatinib treatment among CML patient favoring higher leukemic burden and inferior treatment outcome. Further researches are warranted to evaluate the potential application of GSTP polymorphic variants as pharmacogenetic determinant prior to initiating therapy in order to predict treatment outcome and optimize targeted therapy for CML patients according to their specific genotype.

**Reference**


