RESEARCH ARTICLE

Association of *GSTM1*, *GSTT1* and *GSTP1* Ile105Val polymorphisms with clinical response to Imatinibmesylate treatment among Malaysian Chronic Myeloid Leukemia patients

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ABSTRACT

The detoxifying activity of GST enzymes not only protect cells from the adverse effects of xenobiotics, but also alters the effectiveness of drugs in cancer cells, resulting in toxicity or drug resistance. This current study was aimed to evaluate the association of *GSTM1*, *GSTT1* and *GSTP1* Ile105Val polymorphisms with treatment response among Malaysian Chronic Myeloid Leukemia (CML) patients undergoing 400 mg daily of Imatinibmesylate (IM) therapy. Multiplex Polymerase Chain Reaction was used for the detection of *GSTM1* and *GSTT1* polymorphisms simultaneously and Polymerase Chain Reaction – Restriction Fragment Length polymorphism (PCR-RFLP) analysis was used to detect the *GSTP1* Ile195Val polymorphism.

On evaluating the association of the variant genotype with treatment outcome, heterozygous variant (AG) and homozygous variant (GG) of GSTP1 Ile105Val showed significantly higher risk for the development of resistance to IM with OR: 1.951 (95 % CI: 1.186 – 3.209, P value = 0.009) and OR 3.540 (95 % CI: 1.305 – 9.606, P value = 0.013) respectively. Likewise, *GSTT1* null genotype also was associated with a significantly higher risk for the development of resistance to IM with OR value = 1.664 (95 % CI: 1.011 – 2.739, P value = 0.045). Our results indicate the potential usefulness of GST polymorphism genotyping in predicting the IM treatment response among CML patients.

Keywords: *GSTM1, GSTT1, GSTP1*, Chronic Myeloid Leukemia, ImatinibMesylate,Single Nucleotide Polymorphism

INTRODUCTION

Chronic myeloid leukemia(CML) accounts for 20 % of all cases of leukemias(Baccarani *et al.*, 2015). This clonal myeloproliferative disorder (Karkucak *et al.*, 2012) is characterized by increased levels of leukocytes, splenomegaly, myeloid hyperplasia in bone marrow and also increment of mature myeloid cells in peripheral blood (Kabarowski and Witte, 2000; O'Dwyer *et al.*, 2002; Sawyers, 1999). In CML, a breakpoint cluster region – Abelson (BCR – ABL) oncogene with markedly increased tyrosine kinase activity, is generated from the Philadelphia(Ph) chromosome translocation t (9;22)(q34;q11). An increasing knowledge and advancement of technology in the understanding of the abnormal activity of the BCR-ABL protein had led to the designing and emergence of targeted therapies such as Tyrosine Kinase Inhibitors (TKIs) for Ph chromosome positive CML. ImatinibMesylate (IM) is the first molecularly targeted TKI drug used (Druker *et al.*, 1996) and still remains the gold standard for CML treatment.Despite shown to produce superior results, up to 33 % of CML patients develop resistance to IM. Apart from mutations and amplifications of the BCR-ABL gene, pharmacokinetic variability among patients also accounts for development of drug resistance. It is now becoming increasingly clear that variations in genes involved in

transport, binding and metabolism of IM affect the pharmacokinetics of drugs and might be important determinants of pharmacokinetic variability.

The xenobiotic metabolizing enzymes play important role in the metabolism of drugs including chemotherapeutic drugs. Many of these enzymes are genetically polymorphic. Polymorphisms in genes encoding drug metabolizing enzymes can vary in their enzymatic activity and can play important role in pharmacokinetic variability, potentially modifying treatment response and resistance or drug – related toxicity (Weinshilboum, 2003). Glutathione *S*-tranferases (GSTs) are one of the phase II drug metabolizing enzymes superfamily which are ubiquitous, and multifunctional enzymes and play an important role in cellular detoxification and also in protecting macromolecules from being attacked by the reactive electrophiles (Strange *et al.*, 2001).Out of the eight classes of GSTs, the most commonly studied genes are GSTM, GSTT and GSTP and several types of allelic variations have been identified in them. Polymorphisms occurring within these genes are associated with an increased risk of cancer and diverse response to treatment (Zmorzyński *et al.*, 2015).

GSTμ (GSTM) subfamily encoded by 100kb gene cluster, is located at chromosome 1p13.3 and arranged in order of 5' – GSTM4-GSTM2-GSTM1-GSTM5-GSTM3 – 3' (Landi, 2000; Pearson *et al.*, 1993). *GSTM1* consists of 8 exons in size range of 36 to 112 bp whereas introns vary from 87 to 2641 bp (Parl, 2005). *GSTM1* is embedded and flanked in between two identical, with extensive homologies of 4.2 kb regions. *GSTM1* null allele emerges by homologous recombination of 4.2 kb repeats on right and left resulting in deletion of 16 kb region containing whole sequence of *GSTM1*. Deletion commonly involves both alleles resulting in *GSTM1* - / - and can be technically assessed by PCR assay which will be characterized as null genotype or *GSTM1* - / - when PCR product is absent.

There are two genes existing in Theta-class GST gene cluster (GSTθ) subfamily, separated by about 50 kb, which are *GSTT1* and *GSTT2*, located on chromosome 22q11.2 (Coggan *et al.*, 1998; Landi, 2000; Whittington *et al.*, 1999). The *GSTT1* has five exons

which has size range from 88 to 195 bp and introns which has size range from 205 to 2363 bp (Parl, 2005). *GSTT1* is situated in a region between two 18 kb extensive homology region which have more than 90 % homologies called HA3 and HA5. HA3 and HA5 share 100 % identity of 403 bp sequence. Null deletion of *GSTT1* emerges from the homologous recombination of 403 bp repeats on the left and right which result in 54 kb deletion of whole sequence of *GSTT1*. This null deletion involves only the *GSTT1*, and does not include the *GSTT2*(Coggan et al., 1998).

Glutathione S-transferase P1 (*GSTP1*) which belongs to the pi (π) class gene family, is located at chromosome 11q13 (Autrup, 2000) and spans approximately 2.48 kb and consists of 7 exons (Bora *et al.*, 1997; Morrow *et al.*, 1989). There are two polymorphisms reported and identified in this gene. The first one is A-G polymorphism at nucleotide 313 in exon 5. This polymorphism leads to substitution of amino acid isoleucine (lle) by valine (Val) at 105 amino acid position (lle105Val) (Ali-Osman *et al.*, 1997; Sailaja *et al.*, 2010; Watson *et al.*, 1998). The other polymorphism of this gene is C-T transition at nucleotide 341 which leads to amino acid substitution of alanine by valine at codon 114 in exon 6 (Ala114Val) (Ali-Osman *et al.*, 1997; Hayes *et al.*, 2005; McIlwain *et al.*, 2006).

Genetic variations may result in a decrease in the intracellular enzyme concentration, a dysfunctional protein and may also alter the structure of enzyme, which later leads to changes in the function of the enzyme. Therefore, polymorphisms that decrease the activity of GSTs are associated with an increased risk of cancer development and may also be associated with the phenomenon of drug resistance (Traverso *et al.*, 2013). It was hypothesized that polymorphisms in *GSTM1, GSTT1* and *GSTP1*might be involved in variation in IM metabolism and thus contributing to interindividual variation in IM response in CML patients. The current study was designed to test the above hypothesis.

MATERIALS AND METHODS

Subject Recruitment

This study was started after getting ethical approval from Research and Ethics Committee of UniversitiSains Malaysia [ethical numbers USMKK/PPP/JEPeM [244.3.(4)] and USMKK/PPP/JEPeM [264.3.(8)])and Ministry of Health Malaysia(NMRR-10-1207-7183).In the present study, 278 CML patients (including 132 IM good responders and 146 IM resistant) were successfully recruited from few hospitals in Malaysia including Hospital UniversitiSains Malaysia (HUSM), UniversitiKebangsaan Malaysia Medical Centre (PPUKM), Sime Darby Medical Centre, Hospital Raja PerempuanZainab II (HRPZII) Kota Bharu, Hospital Pulau Pinang, Penang, Hospital Raja PermaisuriBainun, Ipoh and Hospital Umum Sarawak (HUS), Miri. Only Philadelphia (Ph) chromosome positive CML patients who were treated with 400mg IM for at least 12 months and who were negative for BCR-ABL mutation and amplification were recruited.

Multiplex – PCR analysis for detection of*GSTM1* and *GSTT1* null deletion

Three mL of peripheral blood was collected from study subjects (CML patients) after getting written informed consents. Genomic DNA was extracted using commercialized kit, QIAGEN QIAamp® DNA Blood Mini kit (Qiagen.Hilden, Germany), followed by amplification of targeted gene. Multiplex Polymerase Chain Reaction (Multiple – PCR) was used for the detection of null allele deletion of both *GSTM1* and *GSTT1* simultaneously. Three pairs of primers were used simultaneously in which *β – globin* served as a control (shown in **Table 1**). PCR mixture was prepared in total volume of 20 µL including 2X HotStarTaq*Plus* Mater Mix, 0.1 µM each forward and reserve primers for *GSTM1*, *GSTT1* and *B – Globin*, RNase-free water and DNA template. Conditions used for this multiplex – PCR were initial denaturation at 95ºC for 5 minutes, followed by 30 cycles of the following steps: denaturation

at 94°C for 1 minute, annealing at 55.5°C for 1 minute and extension at 72 °C for 1 minute and lastly with final extension at 72 °C for 10 minutes. In order to determine thesuccess of PCR analysis, 3 % Agarose gel was used. Null deletion was detected when no band appeared at specific region.

Table 1 Primer sequences for multiplex PCR reaction

Gene	Primer Sequence	Primer length (bp)	Product size (bp)	Ref
GSTT1	F: 5' – TTCCTTACTGGTCCTCACATCTC – 3'	23	459	(Shahpudin *et al.*, 2011)
	R: 5' – TCACCGGATCATGGCCAGCA – 3'	20		
GSTM1	F: 5' – GAACTCCCTGAAAAGCTAAAGC – 3'	22	219	(Ateş *et al.*, 2005)
	R: 5' – GTTGGGCTCAAATATACGGTGG – 3'	22		
β-GLOBIN	F: 5' – CAACTTCATCCACGTTCACC – 3'	20	260	(Ateş *et al.*, 2005)
	R: 5' – GAAGAGCCAAGGACAGGTAC – 3'	20		

Genotyping of *GSTP1* polymorphism (Ile105Val)

Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR – RFLP) was used for amplification and detection of *GSTP1* polymorphism. One set of primers were used in order to amplify 433 bp targeted gene which were5' – GTAGTTTGCCCAAGGTCAAG – 3' (forward)and 5' – AGCCACCTGAGGGGTAAG – 3' (reverse). PCR mixture was prepared in total of 25 µL which consisted of 1X PCR buffer, 1 µM of Magnesium Chloride ($MgCl_2$), 0.22 µM of dNTPs, 0.4 µM of each primers, 1 U of Go*Taq* DNA Polymerase, and deionized water. PCR cycles consisted of an initial denaturation at 95 ^{0}C for 2 minutes, followed by 30 cycles of 3 steps: denaturation at 94 ^{0}C,

annealing at 57 ^{0}C and extension at 72 ^{0}C for 45 seconds each respectively and lastly, final extension of 72 ^{0}C for 5 minutes. PCR product was electrophoresed on 2 % agarose gel in order to check the success of amplification. PCR products of all samples were cut using *BsmBI*enzyme with incubation period for 1 hour at 55 ^{0}C. Success of RFLP analysis was observed by the presence of 3 different band sizes of 433 bp, 327 bp and 106 bp on 3 % Agarose gel. A homozygous wildtype produced only one band at 433 bp, a heterozygous variant produced 3 bands at 433 bp, 327 bp and 106 bp and a homozygous variant produced 2 bands at 327 bp and 106 bp.

Following genotyping, a few samples from each different genotype category were randomly selected for direct sequencing to confirm that the designed primers amplified the correct targeted sequence of the candidate gene. The samples were sent to the commercial company,First BASE Laboratories SdnBhd (Kuala Lumpur, Malaysia).

Statistical Analysis

The genotype frequencies of the SNPs studied were calculated. The difference in genotype frequencies of the SNPs between IM good responders and IM resistant group of CML patients were compared using the Chi-square test (χ^2). The association of genotypes with CML response was determined using the binary logistic regression analysis and deriving Odds Ratio (OR) with 95 % confidence interval (CI). Statistical tests were two sided and $p < 0.05$ was considered as statistically significant.

RESULTS

This present study successfully recruited 278 CML patients (132 IM good responders and 146 IM resistant) undergoing Imatinibmesylate treatment. Mutiplex – PCR was able to successfully amplify the three pairs of primers for null deletion detection (**Figure 1**). PCR-

RFLP performed with the *BsmBI* enzyme could correctly cut the targeted region on sequence of *GSTP1* as shown in **Figure 2**.

The distribution of genotype frequencies of the SNPs studied among the two groups of CML patients are shown in **Table 2.** Frequency of *GSTM1* wildtype genotype was higher in good response group as compared to resistant group whereas, null genotype was higher in resistant group as compared to response group, but was not statistically significant. *GSTT1* wildtype was significantly higher in good response as compared to resistant group. In contrast, null genotype of *GSTT1* was significantly higher in resistant group as compared to response group. For *GSTP1*, our results showed that AA (Ile) genotype was significantly higher in IM good responders as compared to the IM resistant CML patients [58.3 % vs 39.7 % (p value = 0.002)]. However, heterozygous variant (AG) and homozygous variant (GG) genotypes were significantly higher in IM resistant CML patients which were 49.3 % and 11.0 % respectively as compared to the IM good responders which were 37.1 % and 4.6 % respectively.

The results on the association of polymorphic genotypes with IM response are shown in **Table 2**. Although *GSTM1* null genotype showed lower risk for development for resistance to IM, it was statistically not significant (OR 0.808, 95 % CI: 0.495 – 1.318, p value = 0.393). On the contrary, *GSTT1* showed significantly higher risk for development of IM resistance with OR value 1.664 (95 % CI: 1.011 – 2.739, p value = 0.045). With regard to the association of *GSTP1* Ile105Val with development of resistance to IM, heterozygous variant showed 1.951 fold higher risk for resistance development (95 % CI: 1.186 – 3.209, P value = 0.009) and homozygous variant showed 3.540 fold higher risk for development of resistance to IM (95 % CI: 1.305 – 9.606, P value = 0.013).

For polymorphisms which did not show a significant association with IM response when acting individually, still association may remain possible when the genotypes of these different polymorphisms are combined. So we investigated the combination of

*GSTM1*vs*GSTT1*, *GSTM1*vs*GSTP1* and *GSTT1*vs*GSTP1* and their association with IM response and the results are presented in Table 3. Combination of both *GSTM1* and *GSTT1* deleted genes (*GSTM1* and *GSTT1* null genotypes) and combination of *GSTM1* present and *GSTT1* null genotypes showed higher OR values indicating higher risk for development of IM resistance, but the values were statistically not significant (p value = 0.419 and p value = 0.277). Similarly, combination of null *GSTT1*vs*GSTP1* Ile/Val and null *GSTT1*vs*GSTP1* Val/Val showed significantly higher OR value (OR value of 4.126 and 5.803) which represent higher risk for IM resistance development for carriers of these combination genotypes. In contrast, combination of null *GSTM1*vs*GSTP1* Ile/Ile showed lower OR value thereby indicating a protective role in IM resistance development for carriers of such genotype combinations.

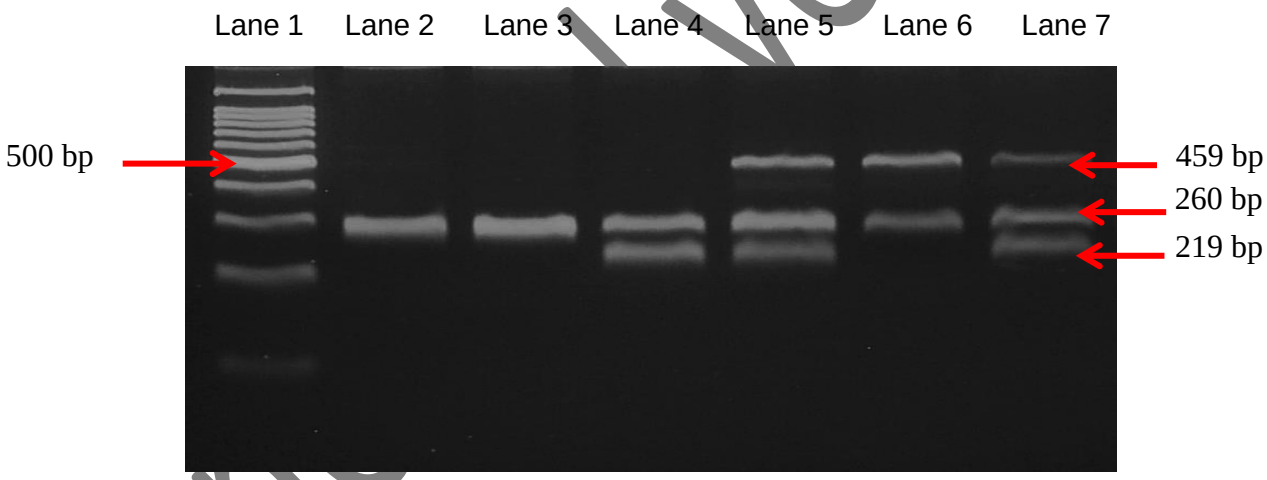

Figure 1 Gel electrophoresis picture showing multiplex – PCR analysis of *GSTT1* and *GSTM1*. Lane 1 represents 100 bp Ladder. Lane 2 and 3 show only one band present at 260 bp (represent deletion of *GSTT1* and *GSTM1*). Lane 4 shows 2 bands present at 260 bp for *β globin* and 219 bp for *GSTM1* (indicates deletion of *GSTT1*). Lane 5 and 7 show presence of three bands at 459 bp (*GSTT1*), 260 bp (*β globin*) and 219 bp (*GSTM1*), representing presence of both genes. Lane 6 shows presence of two bands at 459 bp (*GSTT1*) and 260 bp (*β globin*) indicating deletion of *GSTM1*.

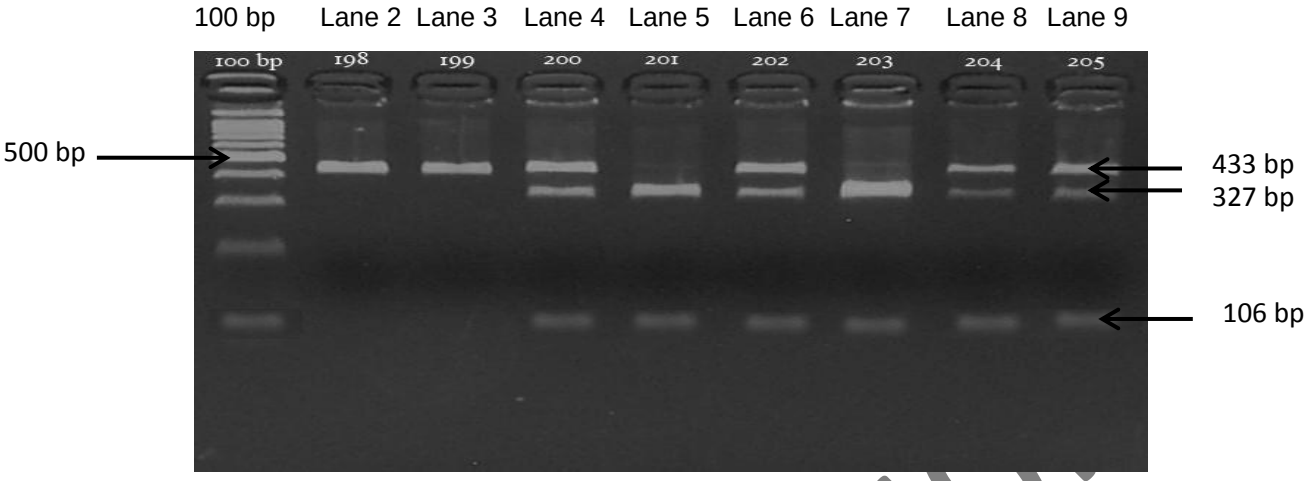

Figure 2 Gel electrophoresis picture showing RFLP analysis of *GSTP1*. Lane 1 represents 100 bp Ladder. Lane 2 and 3 show homozygous wildtype (AA), Lane 4, 6, 8 and 9 show heterozygous variant (AG) and Lane 5 and 7 show homozygous variant (GG).

DISSCUSSION

Drug metabolizing enzymes are involved in the activation and/or detoxification of cytotoxic drugs. Genetic variation in genes encoding for drug metabolizing enzymes could explain interpatient variability in drug response and could be crucial and basal determinants for treatment response assessment. Polymorphisms can result in lack of enzymatic activity and reduced detoxification role for GSTs. Few studies have reported associations of GST polymorphisms with the efficacy and toxicity of cancer chemotherapy and have also been implicated in resistance to some anticancer drugs (Ban *et al.*, 1996; Hayes and Strange, 1995; Tew, 1994). However, reports on the association of polymorphisms in GSTs with response to IM are limited. To the best of available knowledge, this is the first study from Malaysia to determine the association of genetic variations in *GSTP1*, null deletion in both *GSTM1* and *GSTT1* with clinical response to IM in CML patients.

The present study evaluated the association of genetic variation in *GSTM1* and *GSTT1* with IM treatment outcome. Both *GSTM1* and *GSTT1* showed higher frequency of wildtype genotype in IM response group whereas the frequency of null genotype was higher among IM resistant group of CML patients. However, the difference in frequency of *GSTM1* was statistically not significant between the two groups, whereas, *GSTT1* frequency showed significant difference between the two groups (IM resistant and IM good response group). Regarding the susceptibility to IM failure, *GSTT1* null deletion genotype showed 1.6 fold higher risk in development of resistance to IM. This result was concurrent with a study by Ovsepyan et al (2014) among Russian CML patients. Ovsepyan et al (2014) found that carriers of null deletion of *GSTT1* genotype showed 3.3 fold higher risk in failure of achieving cytogenetic response with 95 % CI: 0.75 – 14.47 with p value of 0.013.

The risk of IM treatment failure for those with combined carriership of deletion of *GSTT1* and *GSTM1* was also evaluated. Our study found that carriers of null genotypes of both *GSTT1* and *GSTM1* were higher in IM resistant group as compared to those with good response group, with higher OR values although statistically not significant. This result was concurrent with the result reported by Ovsepyan et al (2014). Lack of statistical significance may be attributed to the small sample size in the present study. According to Ovsepyan et al (2014), the high risk in failure of high cytogenetic response (HCR) after 12 months of imatinib therapy among carriers of *GSTM1* and *GSTT1* null deletion genotypes could be due to increment of incidence in mutations of tumor cells because of less deactivation of exogenous and endogenous genotoxicants, which then lead to promoting the emergence of resistant clones insensitive to imatinib therapy in a daily dose of 400 mg (Ovsepyan *et al.*, 2014). Thus, combined carriership of null deletion of *GSTM1* and *GSTT1* has been implicated as a risk factor for CML progression which then leads to failure of imatinib therapy. This result also highlights the probable significance of gene – gene interactions for the risk of CML progression.

In this present study, frequency of heterozygous variant (AG) of *GSTP1* was significantly higher in IM resistant group as compared to the IM good response group with p value = 0.041. In addition, homozygous variant (GG) also showed elevation in frequency in IM resistant group as compared to the IM good response group with P value = 0.048. We also evaluated the association of genotype pattern of *GSTP1* Ile105Val with the response to Imatinibmesylate and found that carriage of heterozygous variant and homozygous variant genotypes were associated with higher risk for development of resistance towards IM with OR 1.951 (95 % CI: 1.186 – 3.209, P value = 0.009) and OR 3.540 (95 % CI: 1.305 – 9.606, P value = 0.013) respectively. Among Indian CML patients, Sailaja*et a*l., (2010) also had shown results on the response to IM in terms of haematological response which indicated elevation in frequency of combined genotypes in cytogenetic poor (41.6 %) and minor (53.7 %) responders as compared to major (38.51 %) responders. Sailaja *et al.*, 2010 suggested that *GSTP1* Ile105Val polymorphism with reduced *GSTP1* enzyme activity might induce abundance of intermediate metabolites in the body and initiate additional mutation which delays response rates towards treatment and also favors disease progression (Sailaja *et al.*, 2010).

In addition, we also determined the risk for IM treatment failure for combination of *GSTP1*Ile105Val genotype with *GSTM1* deletion as well as *GSTT1* deletion in order to strengthen the finding of this study. In the *GSTM1* null and *GSTP1* genotype combination analysis, the *GSTM1* null / *GSTP1* Ile105Ile genotypes combination showed significant lower risk for development of resistance to IM with p value of 0.028. However, when association of combination genotype of *GSTT1* and *GSTP1* with IM response was assessed, higher risk to IM resistance development was observed among carriers of *GSTT1* null/*GSTP1* Ile105Val and *GSTT1* null/*GSTP1* Val105Val combination genotypes and the associations were statistically significant. Our data could not be compared with other reports as no other reports on combination genotype analysis are available.

Due to the dual role of activation and detoxification, the specific mechanism through which GSTs modify the response to IM treatment cannot be explained properly. According to Marengo et al (2013), GSTs can combine with mitogen activated protein kinases (MAP/MAPK) (Traverso *et al.*, 2013). As a result of this, apoptosis – inducing MAP kinases are blocked and cannot be activated by compounds having anticancer properties. However, the interplay of genetic and cellular environment factors cannot be underestimated while exploring the clinical impact of GST in IM therapy and this remains an issue to be explored.

CONCLUSION

The present study showed that CML patients with carriage of heterozygous and homozygous variant genotypes of *GSTP1* Ile105Val and *GSTT1* null genotype have a higher risk for development of resistance to IM. Hence, genetic variation in GST superfamily genes could be considered as pharmacogenetic markers to predict the treatment outcome for Imatinibmesylate among CML patients.

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Table 2 Genotype frequencies and risk association of *GSTM1, GSTT1* and *GSTP1* Ile105 Val polymorphism with IM response in CML patients

Genotypes	Resistance (n=146) %	Good response (n=132) %	P – value*	OR	95% CI	P – value#
GSTM1						
Present	57 (39.0)	45 (34.1)	0.392	1.000	REFERENCE	-
Null	89 (61.0)	87 (65.9)		0.808	0.495 – 1.318	0.393
GSTT1						
Present	86 (58.9)	93 (70.5)	**0.044***	1.000	REFERENCE	-
Null	60 (41.1)	39 (29.5)		1.664	1.011 – 2.739	**0.045***
GSTP1						
Ile/Ile	58 (39.7)	77 (58.3)	**0.002***	1.000	REFERENCE	-
Ile/Val	72 (49.3)	49 (37.1)	**0.041***	1.951	1.186 – 3.209	**0.009***
Val/Val	16 (11.0)	6 (4.6)	**0.048***	3.540	1.305 – 9.606	**0.013***

*p < 0.05 (statistically significant)

OR Odds Ratio

CI Confidence Interval

*Chi-square test

#Simple logistic regression

Table 3 Risk association of combined genotype of *GSTM1,GSTT1* and *GSTP1* polymorphisms with IM response in CML patients

Combined Genotypes		Resistance (N=146) %	Good Response (N=132) %	OR	95% CI	P - value
GSTM1	***GSTT1***					
PRESENT	PRESENT	36 (24.7)	33 (25.0)	-	-	-
NULL	NULL	39 (26.7)	27 (20.5)	1.324	0.670 – 2.616	0.419
PRESENT	NULL	21 (14.4)	12 (9.1)	1.604	0.684 – 3.761	0.277
NULL	PRESENT	50 (34.2)	60 (45.4)	0.764	0.418 – 1.396	0.382
GSTM1	***GSTP1***					
M1(+/+)	Ile/Ile	28 (19.2)	23 (17.4)	-	-	-
M1(+/+)	Ile/Val	23 (15.8)	17 (12.9)	1.111	0.482 – 2.561	0.804
M1(+/+)	Val/Val	6 (4.1)	5 (3.8)	0.986	0.266 – 3.649	0.983
M1(-/-)	Ile/Ile	29 (19.9)	53 (40.1)	**0.449**	**0.220 – 0.917**	**0.028***
M1(-/-)	Ile/Val	50 (34.2)	33 (25.0)	1.245	0.615 – 2.520	0.543
M1(-/-)	Val/Val	10 (6.8)	1 (0.8)	8.214	0.978 – 69.007	0.052
GSTT1	***GSTP1***					
T1(+/+)	Ile/Ile	49 (37.1)	38 (26.0)	-	-	-
T1(+/+)	Ile/Val	40 (30.3)	40 (27.4)	1.289	0.701 – 2.372	0.414
T1(+/+)	Val/Val	4 (3.0)	7 (4.8)	2.257	0.615 – 8.276	0.220
T1(-/-)	Ile/Ile	27 (20.5)	20 (13.7)	0.955	0.466 – 1.956	0.900
T1(-/-)	Ile/Val	10 (7.6)	32 (21.9)	**4.126**	**1.805 – 9.433**	**0.001***
T1(-/-)	Val/Val	2 (1.5)	9 (6.2)	**5.803**	**1.184 – 28.445**	**0.030***

p> 0.05 (statistically not significant)

OR Odds Ratio

CI Confidence Interval