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Research Article

Human Genetics

The Impact of CYP3A5, CYP1A1, GSTM1, GSTT1, GSTP1 and TPMT Gene Polymorphisms on the Risk of Chronic Myeloid Leukemia

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Abstract: Polymorphisms in the genes encoding drug metabolising enzymes (DME) cause inter-individual variations in metabolising exogenous and endogenous substances, which has been related to the risk of various diseases. The present study was conducted to investigate the association of CYP1A1, GSTM1, GSTT1, GSTP1 and CYP3A5 and TPMT gene polymorphisms with susceptibility to chronic myeloid leukemia (CML). 89 samples of CML patients and 135 control samples were included in this study. Genotyping of CYP1A1, GSTM1, GSTT1, GSTP1 and CYP3A5 and TPMT gene polymorphisms was performed by a Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP). The frequency of GSTM1 null genotype was found to be significantly higher among chronic myeloid leukemia (CML) patients vs controls (39% vs 18.5%, respectively) with a 2.85-fold increased risk for CML. The frequency of GSTT1 null was found to be significantly lower in CML patients vs controls (26% vs 40.7%). Under the dominant inheritance model, GSTP1 gene polymorphism was significantly associated with reduced risk of CML. Moreover, CYP3A5 * 6 and TPMT * 2 alleles were absent among CML patients and the control group. *GSTM1* null genotype was found to be associated with an increased risk of CML whereas TPMT * 2 and CYP3A5 * 6 alleles were absent in the population of Punjab.

Keywords: CML ; Genetic Polymorphism; Metabolizing Enzymes, PCR-RFLP, SNP

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1. INTRODUCTION

We are constantly exposed to various chemicals that can cause genetic changes in hematopoietic precursor cells and lead to leukemia growth[1]. The contribution of a compound in causing cancer or some other disease depends not only on the extent of exposure but also on the effectiveness of the individual's ability to remove toxins from the body involving detoxification enzymes[2]. The detoxification systems are extremely complex, and their ability to eliminate toxins varies greatly depending on an individual's environment and genetic makeup[3]. Transformation of toxic compounds occurs in two phases: Phase I entails the creation of a reactive site through enzymatic activity involving various CYP450 family enzymes, and Phase II involves the conjugation of a water-soluble group to the reactive site through conjugation enzymes such as Glutathione S-transferases (GSTs), resulting in a water-soluble compound that can be easily excreted through urine or bile[4]. Antiporters, which are energy-dependent efflux pumps, pump these compounds out of the cell. The antiporter activity (p-glycoprotein or multidrug resistance, MDR) has also been defined as Phase III detoxification system. The presence of different versions of a gene encoding that activity, or genetic polymorphism, causes genetic differences in the ability to metabolize xenobiotics[5]. Many enzymes are involved in either activation or detoxification of chemical carcinogen[6]. CYP 3A5 gene is a part of clusters of cytochrome P450 genes. Its cytogenetic location is 7q21.1. The gene spans 31,805 bp and consists of 18 exons. It metabolizes more than 50 per cent of drugs currently in use, including antipsychotics (olanzapine), antiestrogen (tamoxifen), anticancer (irinotecan, docetaxel, vincristine, Imatinib), antimalarial (mefloquine, artemether, lumefantrine). Important genetic variants which decrease the expression of CYP3A5 protein are CYP3A5*3 (rs776746;6986A>G), CYP3A5*6 (rs10264272;14690G>A),CYP3A5*7 (rs76293380; 27131–27132insT)[7,8]. Non-functional polymorphic alleles of CYP3A5 have been related to breast cancer, AML, and ALL[9]. Cytochrome P450 (CYP450) belongs to phase I biotransformation enzymes thatmetabolizexenobiotics or pre-carcinogens to DNA reactive metabolites[10]. The major isoforms of the CYP family responsible for metabolic activation of pre-carcinogens are CYP1A1, CYP1A2, CYP1B1, CYP2E1 and CYP3A4[11]. CYP1A1 is involved in activating major classes of pre-carcinogens and is expressed in many epithelial tissues[12]. It is located on chromosome 15q24.1. It encodes aryl hydrocarbon hydroxylase (AHH) enzyme, which is involved in the production of reactive epoxide intermediates from polycyclic aromatic hydrocarbons (PAHs), polyhalogenated aromatic hydrocarbons (PHAHs) etc. PAHs and PHAHs are leading pro-carcinogens found in environmental pollution and may increase the risk of oxidative stress and cancer[13]. It is likely that CYP1A1 gene polymorphism would influence the capacity of an individual with a variant genotype to metabolize different pro-carcinogenic compounds and thus, be a major and important factor determining the individual's susceptibility to develop cancer. *CYP1A1* gene polymorphism includes a T6235C change within the 3' noncoding region of the gene (*2A, forming MspI restriction site). This allele is associated with enhanced enzyme activity[14] and increases the amount of DNA adducts leading to increased risk of cancers. Glutathione S-transferases are one of the major phase-II detoxification enzymes and are characterized into six distinct families: alpha (*GSTA*), mu (*GSTM*), omega (*GSTO*), pi (*GSTP*), theta (*GSTT*) and zeta (*GSTZ*). Among them, functional polymorphisms have been reported for *GSTM1*, *GSTT1* and GSTP1 genes. Homozygous deletions of *GSTT1* and *GSTM1* genes lead to loss of enzyme activity[15] and Val105 form of GSTP1 results from A>G base substitution at nucleotide 313 which maybe 2-3 times less stable than Ile105 form (wild type)[16]. Inherited differences in the capacity of these enzymes to metabolize xenobiotics might be an important genetic factor leading to susceptibility to cancer[17]. The TPMT gene is located on chromosome 6p22.3. It has a length of 26,833 bp and nine exons. The use of TPMT in the metabolism of thiopurine drugs including azathioprine, 6-mercaptopurine, and 6-thioguanine, is well-known[18]. S-methylation of thiopurine drugs is catalysed by TPMT. Defects in the TPMT gene cause decreased methylation and inactivation of 6MP, which causes myelosuppression, anaemia, bleeding propensity, leukopenia, and inflammation in the bone marrow[19]. There are 35 TPMT genetic polymorphisms \associated with decreased TPMT enzyme activity level and thiopurine drug induced toxicity[20]. These polymorphic variants are considered as pharmacogenetic markers which enable the individualization of thiopurine drug therapy. Ninety percent of individuals inherit both functional TPMT alleles resulting in high TPMT activity. Patients with low TPMT activity are at high risk of severe, eventually fatal, haematologic toxicity. Among the 35 TPMT polymorphic variants, the most common are: TPMT*2 (rs1800462;238G>C), TPMT*3B (rs1800460;460G>A) and TPMT*3C(rs1142345; 719A>G) which are associated with the thiopurine drug-related toxicity [21].Since no such study has been reported in the Punjab region of India, The aim of this study was to identify the role of CYP1A1, GSTM1, GSTT1, GSTP1, CYP3A5, and TPMT gene polymorphisms in the risk of CML in the Punjab state population in North India.

2. MATERIALS AND METHODS

2.1 *Subjects*

The present case-control study involved chronic myeloid (CML) patients (N=89) and healthy controls (N=135). The patients were recruited after pathological confirmation from Sandhu Cancer Centre, Ludhiana. Peripheral blood samples were collected in EDTA coated vials. This study was approved by the Institutional Ethical Research Committee(ICEC/12/2011). Written informed consents were obtained from cases and controls.

2.2 *Genotyping*

Genomic DNA used for genotypic analysis was isolated from blood samples using the inorganic (salting out) method[22]. Table 1 lists the markers studied, primer sequences and the restriction enzymes used.

2.3 *CYP3A5*6 polymorphism*

After an initial denaturation step at 94C for 5 min, annealing at 53˚C for 10sec, extension at 72˚C for 10sec, and final extension at 72˚C at 5 min. The protocol was standardized and optimized for all the parameters like varying annealing temperature, dNTPs, primer, MgCl2, *Taq*polymerase and template concentration. Genomic DNA 50- 150ng was amplified in a total volume of 25µl reaction mixture containing 0.2mM dNTPs, 0.4pmoles/µl of each primer and

1.5U of *Taq*polymerase in 1X buffer as shown in Table 1. The reaction mixture was

Table 1. PCR primers and method of analysis for the markers studied

Gene	Gene Variation	Forward and reverse primers	Technique	Size of PCR product (bp)	Ref
GSTM1	Gene deletion	(F) 5'-GAA CTC CCT GAA AAG CTA AGC-3' (R) 5'-GTT GGG CTC AAA TAT ACG GTG-3'	Multiplex PCR	219	Sharma *et al*, 2012
GSTT1	Gene deletion	(F) 5'-TTCCTTACTGGTCCTCACATCTC-3' (R) 5'-TCACCGGATCATGGCCAGCC-3'		459	
Albumin	Positive control	(F) 5'-GCCCTCTGCTAACAAGTCCTAC-3' (R) 5'-GCCCTAAAAAGAAAATCGCCAATC-3'		350	
GSTP1	313 A>G (rs1695) Exon 5	(F) 5'-ACCCCAGGGCTCTATGGGAA-3' (R) 5'-TGAGGGCACAAGAAGCCCCT-3'	PCR-RFLP using *BsmAI* restriction enzyme	176	Harries *et al*, 1997
CYP3A5	14690G>A (rs10264272) Exon 2	(F) 5'-GAGAGAAATAATGGATCTAAGAAAcc -3' (R) 5'- GATAGTTCTGAAAGTCTGTGGC -3'	PCR-RFLP using *Ddel*restriction enzyme	268	Surekha et al., 2009
CYP1A1	6435 T>C (rs4646903) 3' non-coding region	(F) 5'-CAGTGAAGAGGTGTAGCCGCT-3' (R) 5'-TAGGAGTCTTGTCTCATGCCT-3'	PCR-RFLP using *MspI* restriction enzyme	340	Chen *et al*, 2008
TPMT	238G>C (rs1800462)	P2W GTATGATTTTATGCAGGTTTG P2M GTATGATTTTATGCAGGTTTC P2C TAAATAGGAACCATCGGACAC	Allele specific	254 bp	Zhang et al., 2004

gently mixed and placed in a twenty five well automated thermal cycler. The whole reaction was prepared at 4° C. The primer pair used for amplification purpose was designed to generate a Ddel endonuclease restriction site[23]. The amplified product (268 bpfragment) was digested with one unit of Ddelenzyme (New England Biolabs) at 37°C for an hour and electrophoresed on 2.5% Gel. CYP3A5*1 wild allele produces fragments of different sizes 120, 103, 25 &20(Figure 1) and CYP3A5*6 mutant allele produces 128, 120 & 20 bp fragments.

2.4 *CYP1A1*2A polymorphism*

*CYP1A1*2A gene polymorphism was determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP). After an initial denaturation at 95°C for 5 min, amplification was carried out for 30 cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, followed by a final extension at 72°C for 5 min. The PCR product (340bp) was then digested with *MspI* (NEB)[24] in a total volume of 15ul and products were separated by electrophoresis in 3% agarose gel containing ethidium bromide. The bands were visualized under UV transilluminator. Digestion of the PCR product with *MspI* enzyme at the restriction site resulted in an undigested product of 340bp for TT genotype, three fragments of 340bp, 200bp and 140bp for TC genotype and, two fragments of 200bp and 140bp for CC genotype(Fig 2).

2.5 *GSTT1 and GSTM1 polymorphisms*

GSTM1 and *GSTT1* genotypes were determined by multiplex PCR using three sets of primers to amplify fragments of 219bp, 459bp and 350bp for *GSTM1*, *GSTT1* and Albumin gene (internal control), respectively[25]. In all lanes, a 350 bp product corresponding to the Albumin gene product serves as an internal positive control. A 219-bp product indicates the presence of at least one GSTM1 non-null allele. In contrast, 459-bp items indicate the presence of at least one GSTT1 non-null allele. The absence of a GSTM1 or GSTT1 product indicates that the gene is homozygous . PCR reaction was performed in 25ul reaction volume using 2X Taq master mix (NEB), primers and genomic DNA in Eppendorfmastercycler. After an initial denaturation at 95°C for 5 min, amplification was carried out for 35 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were separated by electrophoresis in 2% agarose gel stained with ethidium bromide (Figure 3).

2.6 *GSTP1 polymorphism*

GSTP1 polymorphism was determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP). After an initial denaturation at 95°C for 5 min, amplification was carried out for 30 cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, followed by a final extension at 72°C for 5 min. The PCR product (176bp) was then digested with BamH1 (NEB)[26] in a total volume of 15ul and products were separated by electrophoresis in 3.5% agarose gel containing ethidium bromide. The bands were visualized under UV transilluminator. Digestion of the PCR product with smA1 enzyme resulted in an undigested product of 176bp for Ile/Ile genotype, three fragments of 176bp, 91bp and 85bp for

Ile/Val genotype and, two fragments of 91bp and 85bp for Val/Val genotype (Figure 4).

*2.7 TPMT *2 polymorphism*

An allele-specific PCR was used to analyze the 238G>C (TPMT *2) mutation in exon 5, using sequence specific primers (Table 1). Two separate reactions were performed for each subject; one for the wild type genotype using (P2W) and (P2C) primers. The other is mutation specific using (P2M) and (P2C)[27]. PCR amplifications were carried out in 20 ul final volumes containing 0.5 uM of each primer, 5 ul DNA, 3 ul H2O and 10 ul 2X master mix. The cycling conditions consisted of initial denaturation at 94°C for 2 minutes followed by 35 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 2 minutes(Figure 5).

3. STATISTICAL ANALYSIS

All computations were done by using Statistical Package for Social Sciences (SPSS) software version 16.0. The allele frequencies were tested for the Hardy-Weinberg equilibrium (HWE) for both patients and controls using χ^2 test. Association between chronic myeloid leukemia (CML) risk and genetic polymorphisms of the studied detoxification enzymes were assessed by odds ratio (OR) at 95% confidence interval. The level of significance for comparison was set as $p<0.05$.

4. RESULTS

The distribution of genotypes in all the samples was found to be in Hardy-Weinberg equilibrium. Among patients, 33 (37%) were females and 56 (63%) were males with an average age of 50.09 years. The control group included 50 (37%) females and 85 (63%) males with an average age of 51.01 years. The distribution of CYP3A5*6, *CYP1A1*2A, *GSTM1* null, *GSTT1* null, GSTP1 (A313G) and *TPMT* genotypes among CML patients and controls is summarized in Table 2. No TPMT*2 and CYP3A5*6 *3C alleles were detected among CML patients and controls. The frequencies of TT, TC and CC genotypes of *CYP1A1*2A* gene polymorphism were 56% vs 59%, 29% vs 33% and 15% vs 8% in patients vs controls, respectively. No significant association of *CYP1A1*2A* allele was observed with CML risk in the studied subjects ($p>0.05$). For GST gene polymorphisms, *GSTM1* null, *GSTT1* null and GSTP1 (AG, GG) genotypes were 39% vs 18.5%, 26% vs 40.7% and 38%, 4% vs 42.9%, 9% in patients vs controls, respectively. Individuals having *GSTM1* null genotype were at 2.85-fold increased risk for CML (OR=2.85, 95% CI=1.55-5.24, p=0.0007). *GSTT1* null genotype was associated with reduced risk for CML as there was a significant decrease in the frequency of *GSTT1* null genotype among CML patients compared to controls (p=0.023). We did not find any association of GSTP1 gene polymorphism at genotypic and allelic level with risk to CML, but under the dominant inheritance model (AA vs AG+GG), a significant reduced risk was observed (OR=0.51, 95% CI=0.27-0.96, p=0.034). (Table 3).

Table 2. Comparison of genotype and allele frequency distribution of *GST, CYP3A5, CYP1A1 and TPMT* gene polymorphisms among chronic myeloid leukemia (CML) cases and controls

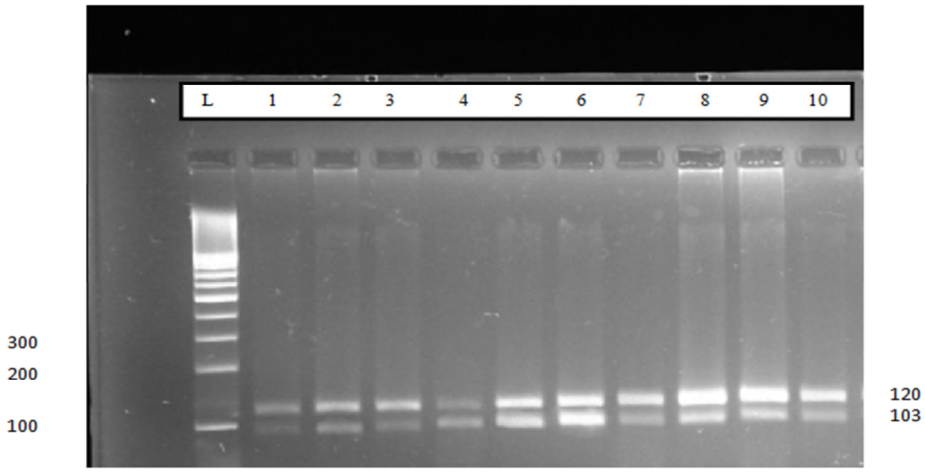
Genotype		Controls (N=135) n (%)	CML (N=89) n (%)	OR (95%CI)	P-value
GSTM1	Non-null	110 (81.5)	54 (61)	Ref	0.0007*
	Null	25 (18.5)	35 (39)	2.85 (1.55-5.24)	
GSTT1	Non-null	80 (59.3)	66 (74)	Ref	0.023*
	Null	55 (40.7)	23 (26)	0.51 (0.28-0.91)	
GSTP1 rs1695 (313 A>G)	AA	65 (48.1)	52 (58)	Ref	
	AG	58 (42.9)	34 (38)	0.73 (0.42-1.28)	0.27
	GG	12 (9)	3 (4)	0.31 (0.08-1.16)	0.08
	A	188 (69.6)	138 (78)	Ref	0.06
	G	82 (30.4)	40 (22)	0.66 (0.43-1.03)	
*CYP3A5*6 (rs10264272; 14690G>A*	GG	135 (100)	89 (100)		
	GA	0 (0)	0 (0)	--	-
	AA	0 (0)	0 (0)	-	-
	G	270 (100)	178 (100)		
	A	0 (0)	0 (0)	-	--
*CYP1A1*2A* (3801 T>C) rs4646903	TT	80 (59)	50 (56)	Ref	
	TC	44 (33)	26 (29)	0.95 (0.52-1.72)	0.85
	CC	11 (8)	13 (15)	1.89 (0.79-4.5)	0.15
	T	204 (75.6%)	126 (71%)	Ref	0.26
	C	66 (24.4%)	52 (29%)	1.27 (0.83-1.95)	
TPMT*2(238G>C) (rs1800462)	GG	135 (100)	89 (100)		
	GC	0 (0)	0 (0)	--	-
	CC	0 (0)	0 (0)	-	-
	G	270 (100)	178 (100)		--
	C	0 (0)	0 (0)	--	

p<0.05

Table 3. Analyses of GSTP1 313A>G and *CYP1A12A polymorphisms with CML risk using different genetic models**

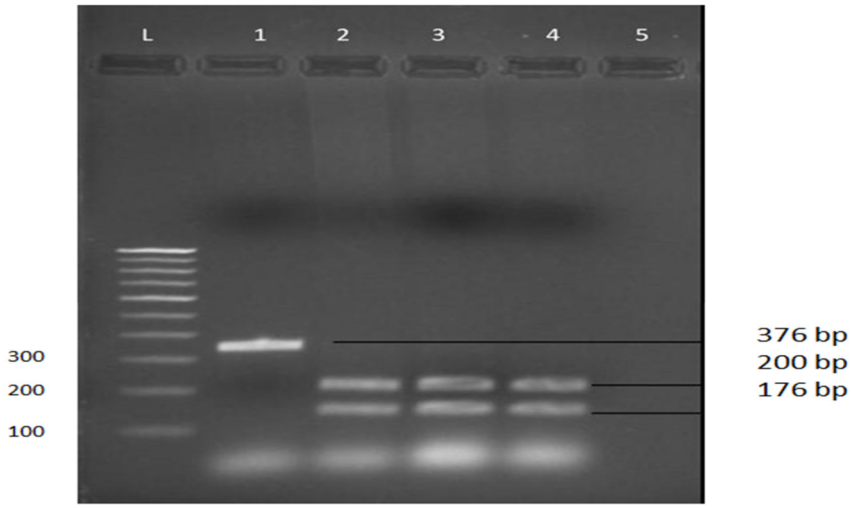
Polymorphism	Model	Genotypes	Cases n (%)	Controls n (%)	OR (95% CI)[a]	P value
GSTP1 313 A>G	Dominant	AA	52 (58.4)	65 (48.1)	Ref	0.034*
		AG+GG	37 (41.6)	70 (51.9)	0.51 (0.27-0.96)	
	Recessive	AA+AG	86 (96.6)	123 (91.1)	Ref	0.11
		GG	3 (3.4)	12 (8.9)	0.32 (0.07-1.45)	
	Overdominant	AA+GG	55 (61.8)	77 (57%)	Ref	0.17
		AG	34 (38.2)	58 (43%)	0.64 (0.34-1.21)	
*CYP1A1**2A 3801 T>C	Dominant	TT	50 (56.2)	80 (59.3)	Ref	0.25
		TC+CC	39 (43.8)	55 (40.7)	1.45 (0.77-2.71)	
	Recessive	TT+TC	76 (85.4)	124 (91.8)	Ref	0.15
		CC	13 (14.6)	11 (8.2)	2.10 (0.77-5.73)	
	Overdominant	TT+CC	63 (70.8)	91 (67.4)	Ref	0.78
		TC	26 (29.2)	44 (32.6)	1.10 (0.57-2.14)	

p<0.05



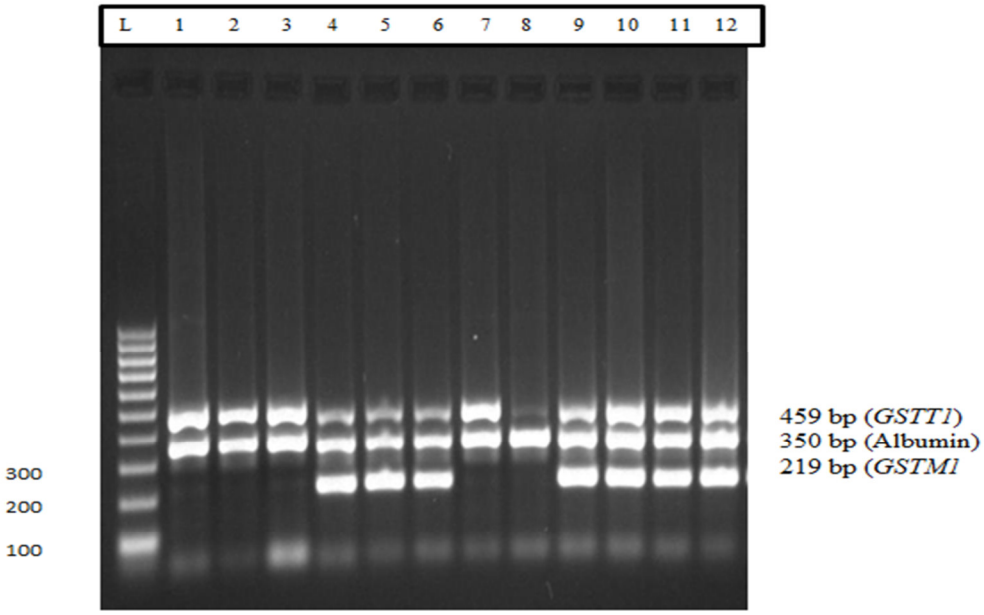
Lane 1- 100 bp DNA Ladder
Homozygous wild (GG) (120bp, 103bp) (1,2,3,4,5,6,7,8,9,10)
Restriction enzyme used: DdeI

Fig 1.Agarose gel showing genotype of CYP3A5 gene polymorphism by PCR-RFLP



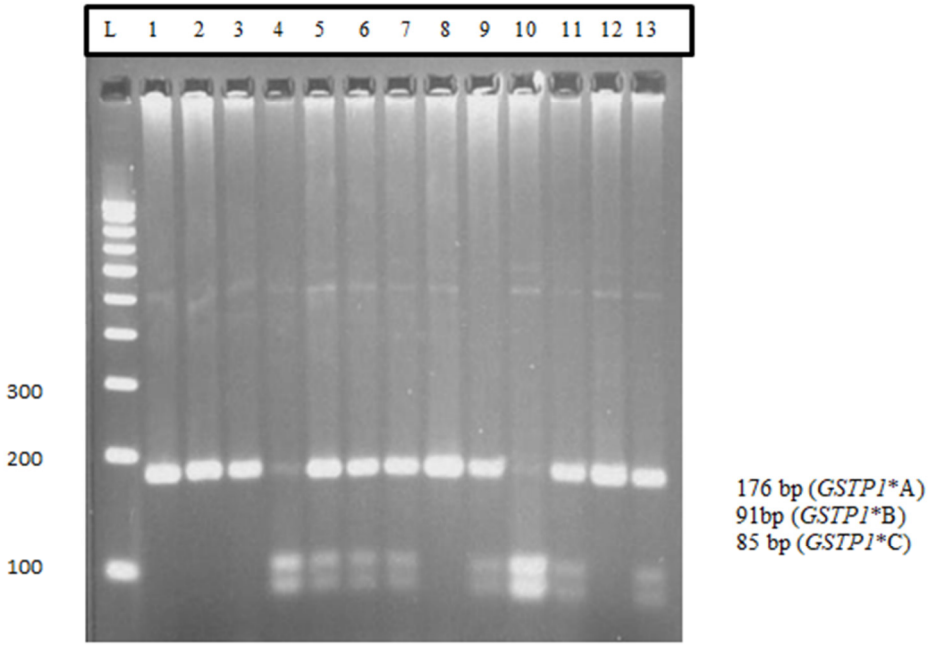
L- 100 bp DNA LADDER
Homozygous Wild (TT) : Lane 1 (376 bp)
Homozygous Mutant (CC): Lane 2,3,4 (200 bp, 176 bp)
Restriction enzyme used: MspI

Fig 2. CYP1A1 polymorphic genotypes resolved by Agarose gel (3%) electrophoresis



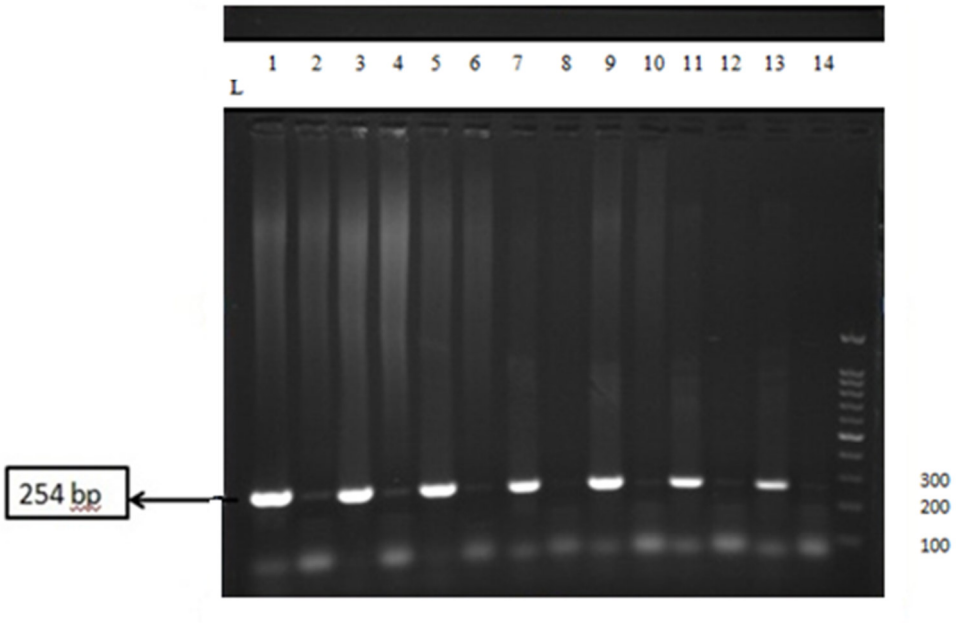
L- 100 bp DNA LADDER
LANE 1, 2, 3, 7—GSTM1 Null Genotype
LANE 4, 5, 6, __ GSTT1 Null Genotype
LANE 8 __ GSTM1, GSTT1 Null Genotype
LANE 9,10,11,12--- GSTM1, GSTT1 Normal Genotypes

Fig 3.*GSTM1* and *GSTT1* genotypes resolved using 2% Agarose gel electrophoresis



L- 100 bp DNA LADDER
Restriction digestion (BsmAI) profile:
AA (homozygous wild type): 176bp (Lane: 1, 2, 3, 8, 9, 12)
AG (heterozygous): 176bp, 91bp, 85bp (Lane: 5, 6, 7, 11, 13)
GG(homozygous mutant): 91bp, 85bp (4, 10)

Fig 4.*GSTP1* genotypes resolved using 4% Agarose gel electrophoresis



L- 100 bp DNA LADDER
Allele specific- PCR
GG (homozygous wild type): 254bp (Lane: 1, 3, 5, 7, 9, 11, 13)

Fig 5.*TPMT2 (rs1800462; 238G>C) gene polymorphism resolved by agarose gel (2%) electrophoresis**

5. DISCUSSION

Patients with CML have a Ph chromosome (Philadelphia chromosome) formed by a translocation between chromosomes 9 and 22. This translocation results in the fusion of bcr-abl gene, which results in increased tyrosine kinase activity and is considered responsible for the development of CML[28]. It is likely that environmental exposure to cytotoxic and genotoxic agents (xenobiotics) may be associated with the initiation of neoplastic processes and thus, increase the risk of CML[29]. Xenobiotics are processed by two phases of biotransformation reactions which include absorption, distribution, metabolic activation and elimination (ADME). Transformation of toxic and carcinogenic xenobiotics to their non-toxic forms is an important step towards their subsequent elimination. In phase I, the CYP450 enzyme family activates toxic and carcinogenic substances and in phase II, enzymes like GSTs detoxify these substances by converting them into easily excretable hydrophilic compounds[30]. These hydrophilic compounds are subsequently expelled out of the cell by P-glycoprotein (P-gp) encoded by *MDR1* gene present in the plasma membrane. Individuals carrying variant alleles resulting in higher activity of enzymes involved in the activation of carcinogens (phase I) and/ or less efficient variant alleles responsible for low or nil activity of detoxification enzymes (phase II) and variant alleles responsible for low activity of P-gp are at greater risk of developing malignancies [31,32]. In the present study, we report the genotypic distribution of CYP3A5*6, *CYP1A1**2A, GST (M1, T1 and P1 313A>G) and TPMT*2 polymorphic variants among CML cases and controls, and their comparison. In the present study, CYP3A5*6 allele was absent in both of the CML patients and controls. These correspond with the studies from Japanese population[33] and Caucasian[34]. In Indians, CYP3A5*3 is the only variant allele present in North Indians (NI) and South Indians (SI). CYP3A5*6 were absent in Indians[35,36]. TPMT*2 polymorphism was absent in the population of Punjab. These results are consistent with the studies conducted by Kenya[37], Egyptian, South West Asian [38], Norwegian Caucasian[39] and Thai[40]. Besides this, the TPMT*2 variants were found at a low allele frequency of 0.20 and 0.10 among American Caucasian[41] and Swedish[42] in comparison to Indian (0.61)[43], Brazilian (0.80)[44] and French Caucasian (3.00)[45]. In our study, the frequency of variant genotype of *CYP1A1**2A was higher in CML cases than controls but this difference was not statistically significant. To the best of our knowledge, only one such study has been reported on the investigation of *CYP1A1* polymorphism in CML patients[46]. In this study, rather a significant reduced risk of CML with *CYP1A1**2A allele has been reported. In a meta-analysis[47], it was reported that *CYP1A1**2A allele is significantly associated with increased risk to AML. However, many studies have been reported the association of *CYP1A1**2A polymorphism with susceptibility to acute leukemia and solid tumors. Other variants of *CYP1A1,* such as *CYP1A1**2C have been studied in CML patients [48,49,50.] Regarding the GST genes, we found that individuals with *GSTM1* null genotype were at increased risk of developing CML. A total of about 15 studies of which 5 were from India have been published on the relationship of GST gene polymorphism with chronic myeloid leukemia (CML). Results of our study were similar to results[51], which reported a significantly elevated frequency of *GSTM1* null genotype among CML patients from Brazilian population. However, results of many other studies were in contradiction to these findings [52,53,54,55,56,57] including Indian studies [58,59,60]. In contrast to our study, all the above mentioned Indian studies reported significant association of *GSTT1* null genotype with susceptibility to CML. However, we found that *GSTT1* null genotype was associated with significant reduced risk to CML. Variable combinations of susceptibility gene variants in individuals could also be an important factor in determining the susceptibility to disease. To the best of our knowledge, only four studies were observed [61,62,63,64] demonstrating the effect of GSTP1 Val allele among CML cases from India, Turkey and Egypt, respectively. Sailaja*et al.* (2010), Dunna*et al.* (2012) as well as Elhoseiny*et al* (2014) reported significantly higher GSTP1

Val/Val genotype frequencies among CML patients as compared to controls. However, a comparison of allele frequencies did not show any significant results. Karkucak*et al* (2012) however, did not find any significant association. Our study also did not report any GSTP1 variant genotypic or allelic association with CML.

6. CONCLUSION

In conclusion, our study showed a significant correlation of *GSTM1* and *GSTT1* genes in the etiology of CML. *GSTM1* null genotype suggests a significant association with an increased risk to CML whereas, *GSTT1* null variant genotypes play a protective role in developing CML. No TPMT*2 and CYP3A5*6 alleles were detected. The significance of our study lies in the fact that this is the first study reporting the baseline data of the polymorphisms of six genes among the CML patients and controls from the population of Punjab.

7. ACKNOWLEDGMENT

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8. AUTHORS CONTRIBUTION STATEMENT

MsShruticaplash conceptualized and gathered the data with regard to this work. MrKaishivjoshi and MsShruticaplash performed the laboratory analysis and analyzed the data using statistical tools. MrKaishivjoshi and MsShruticaplash discussed the methodology and results of the manuscript and discussions part of the manuscript was compiled by Mr. Kaishivjoshi and Dr. Satbirkaur. All the authors read and approved the final version of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

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