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RESEARCH ARTICLE

Role of Antioxidant Gene Polymorphisms in Risk and Prognosis of Chronic Myeloid Leukemia

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Abstract

Introduction: We aimed to investigate the possible role of antioxidant enzyme polymorphisms *CAT* -21A/T (rs7943316), *CAT* -262C/T (rs1001179), *GPX1* -198C/T (rs1050450), *MPO* -463G/A (rs2333227), *GSTM1* (rs366631) & *GSTT1* (rs17856199) with susceptibility to chronic myeloid leukemia (CML) and their association with tyrosine kinase inhibitor (TKI, imatinib) response. **Methods:** Six single nucleotide polymorphisms (SNPs) in antioxidant enzyme genes were genotyped in a total of 325 samples, of which 125 were from CML patients and 200 from healthy controls. The SNPs were correlated with various confounding variables lke *BCR-ABL1* levels and tyrosine kinase domain mutation status in CML patients. **Results:** Genotyping results revealed statistically significant associations with *CAT* -21A/T (p=0.037) and *GPX1* -198C/T (p=<0.0001) polymorphisms with risk of CML. No associations were observed between *CAT* -262C/T, *MPO* -463G/A, *GSTM1* & *GSTT1* polymorphisms and CML. The *CAT* -21A/T polymorphism conferred 2.95 folds increased risk of CML under co-dominant model (p=0.024) and 2.51 folds risk under dominant models (p=0.05). In addition, the haplotypes of *CAT* -21A/T and -262C/T polymorphisms, ATCC and ATCT conferred higher incidence of CML risk by 2.67 times (p=0.05) and 2.99 times (p=0.045). The *GPX1* -198C/T polymorphism conferred significantly increased risk of CML under co-dominant model [CC vs CT (p=<0.0001), CC vs TT (p=<0.0001)] and dominant models [CC vs CT+TT (p=<0.0001)]. The heterozygous *GPX1* CT genotype frequency significantly elevated in poor molecular responders (p=0.005) and TKD mutation carriers (p=0.114) as compared to respective groups. **Conclusions:** Our results suggest that the reduced activity of antioxidant enzymes caused by the *CAT* -21A/T and *GPX1*-198C/T polymorphisms might contribute to increased risk of CML. In addition, the *GPX1*-198C/T polymorphism was associated with poor molecular response and acquired TKD mutations. Hence, the present study indicates that defective antioxidant defense system might have a strong influence on CML susceptibility and TKI (imatinib) response through oxidative stress.

Keywords: Chronic Myeloid leukemia- Imatinib- Resistance- Antioxidant genes- polymorphism

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Introduction

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder of myeloid precursors, characterized by the presence of Philadelphia (Ph) chromosome, which results from a reciprocal chromosomal translocation t (9;22), leading to the *BCR-ABL1* fusion gene. The *BCR-ABL1* gene can modulate DNA repair mechanisms, cell cycle checkpoints, *Bcl2* proteins and enhances reactive oxygen species (ROS) generation, which may contribute to genomic instability and resistance towards tyrosine kinase inhibitor (TKI) treatment [1-3].

The intrinsic regulation of ROS is one of the mechanisms associated with multidrug resistance and maintenance of cancer stemness [4-5]. Previous studies have demonstrated that oxidative DNA damage induced by higher levels of ROS has been associated with initiation and progression of solid tumors and hematological

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malignancies [6-9]. Antioxidant enzymes such as catalase (*CAT*), manganese superoxide dismutase (*MnSOD*), glutathione peroxidase 1 (*GPX1*), myeloperoxidase (*MPO*) and glutathione-S-transferases (*GSTs*) balance ROS levels and defend cells against oxidative stress. Most of these antioxidant enzymes are highly polymorphic. Genetic variations of these antioxidant enzymes with altered enzymatic activity may contribute to the imbalance of ROS production and scavenging [10-11]. The activity of several antioxidant enzymes was noted to be reduced in CML patients [12]. Several studies demonstrated that polymorphisms in antioxidant enzymes (*CAT, MnSOD, GPX1, MPO & GSTs*) might be associated with susceptibility to various solid tumors [13-15] and hematological malignancies [16-20].

Hence, the present study aimed to investigate the possible role of polymorphisms in antioxidant enzyme polymorphisms: Catalase *(CAT)* -21A/T & -262C/T, Glutathione peroxidase 1 (*GPX1*) -198C/T, Myeloperoxidase (*MPO*) -463G/A, deletion of Glutathione S-Transferase M1 & T1 (*GSTM1 & GSTT1*) with susceptibility to chronic myeloid leukemia and their association with TKI (imatinib) response.

Materials and Methods

The present study included 325 samples, out of which 125 are from CML patients and 200 were from age & gender matched controls without a family history of any cancer. The inclusion criteria for patients included Ph+ve CML cases with confirmed diagnosis, on TKI treatment and TKI refractory cases regardless of age, gender or race. The study was approved by the institutional ethics committee and an informed consent was obtained from patients participating in the study. Blood samples (6mL in EDTA vaccutainer) were collected from both CML patients and controls. Genomic DNA was extracted from blood samples using non-enzymatic rapid salting-out method. The purity & concentration of DNA samples were checked on Nanodrop1000 and further these DNA samples were subjected for analysis of SNPs in antioxidant enzyme genes.

Genotyping of antioxidant gene SNPs

Genotyping of *CAT* -21A/T (rs7943316), *CAT* -262C/T (rs1001179), *GPX1* (-198C/T rs1050450) and *MPO* (-463G/A rs2333227) was performed by PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) method. The null/deletion polymorphism in *GSTM1 & GSTT1* genes (rs366631 & rs17856199) were performed by multiplex polymerase chain reaction followed by agarose gel electrophoresis. The primers used for amplification and restriction enzymes for RFLP analysis are listed in Table 1. The *CAT* (-21A/T & -262C/T), *GPX1* (-198C/T) and *MPO* (-463G/A) polymorphism were determined by digesting the PCR amplified products with *HinfI, SmaI, ApaI and SsiI* restriction enzymes (Table 1).

Statistical analysis

Chi square and multivariate analysis tests were calculated to test the significance of genotype association with the occurrence of CML and its prognosis. All the p values were two sided and the level of significance was taken as $p < 0.05$. Statistical analyses were performed using the GraphPad Prism software version 6.0 (San Diego, CA) and online VassarStats software. Haplotype and pairwise linkage disequilibrium was calculated using Haploview version 4.2 and cox regression analysis by SPSS version 22 software.

Results

Baseline characteristics (Table 2)

The demographic and clinical characteristics of CML patients are presented in Table 2. The median age at diagnosis of CML was 42 years (range 12 to 89 years) and a male preponderance was observed with a male to female ratio of 1.6:1. Of the 125 patients, 102 cases presented in chronic phase, 13 in accelerated phase and 10 in blast crisis phase of CML.

Prognostic scores like Sokal, Hasford, and EUTOS (European Treatment Outcome Study) were calculated for all patients using baseline hematological variables [21]. With Sokal risk scoring, 37.6% of patients had low risk and 62.4% had intermediate + high risk. With respect to Hasford risk score, 39.2% had low risk and 60.80% had intermediate + high risk. When EUTOS risk scores were considered, 72.0% of patients were presented with low risk and 28% with high EUTOS risk. Majority of patients were on imatinib (IM) treatment, nearly 42.4% of patients received higher IM doses (600mg/ 800mg), 16.8% on IM standard dose (400mg), 16.0% on other drugs (2^{nd} generation TKIs or on clinical trials), 16.8% deceased and 8% are newly diagnosed.

Median follow-up of these patients for a period of 40 median months revealed that 20.8% had optimal response to imatinib and 79.02% of patients lost respone which might be either due to loss of complete hematological response (CHR), complete cytogenetic response (CCyR), major molecular response (MMR) or presence of TKD mutations.

Correlation with CAT -21A/T polymorphism (Table 3)

The *CAT* -21A/T genotyping results revealed that heterozygous AT genotype frequency was observed to be significantly increased in CML patients compared to controls (p=0.037). This polymorphism was significantly asscciated with increased risk of CML. With respect to molecular response, homozygous TT genotype and T allele frequencies were elevated in non-responders i.e., patients having higher *BCR-ABL1* expression levels (44.70%, 0.705) compared to responders i.e., patients having lower levels (35.0%, 0.625) (p=0.259). Heterozygous AT genotype frequency was found to be slightly increased in TKD mutation carriers (p=0.571) and in deceased group of patients (p=0.548) when compared to respective groups. No differences were found with either of the prognostic risk scores: Sokal, Hasford or EUTOS.

The *CAT* -21A/T polymorphism showed statistically significant association with risk of CML and conferred

Table 1. Primer Sequences used for Analysis of Polymorphisms in Anti oxidant Enzyme Genes

Gene	SNP	Primer sequence	Product size	Restriction enzyme
CAT	-21A/T	5′- AATCAGAAGGCAGTCCTCCC-3′	250bp	HinfI
	(rs7943316)	5′- TCGGGGAGCACAGAGTGTAC-3′		
CAT	-262C/T	5′- AGAGCCTCGCCCCGCCGGACCG-3′	185bp	SmaI
	(rs1001179)	5′- TAAGAGCTGAGAAAGCATAGCT-3′		
GPX	-198C/T	5′- TCCAGACCATTGACATCGAG-3′	222bp	ApaI
	(rs1050450)	5′- ACTGGGATCAACAGGACCAG-3′		
MPO	-463G/A	5′- CGGTATAGGCACACAATGGTGAG-3′	350bp	SsiI
	(rs2333227)	5′- CAATGGTTCAAGCGATTCTTC-3′		
GSTM1	Deletion	5′- GAACTCCCTGAAAAGCTAAAGC-3′	219bp	
	(rs366631)	5′- GTTGGGCTCAAATATACGGTGG-3′		
GSTT1	Deletion	5′- TTCCTTACTGGTCCTCACATCTC-3′	480bp	
	(rs17856199)	5′- TCACCGGATCACGGCCAGCA-3′		
Beta globin		5′- ACACAACTGTGTTCACTAGC-3′	299bp	
		5′- CAACTTCATCCACGTTCACC-3′		

2.95 folds increased risk of CML under codominant (AA vs AT) model (OR=2.953, 95% CI: 1.206-7.228, p=0.024) and 2.51 folds risk under dominant (AA vs AT+TT) models (OR=2.518, 95% CI: 1.058-5.992, p=0.05), whereas overdominant model (AT vs AA+TT) was found to be protective against CML (OR=0.632, 95% CI: 0.404-0.994, p=0.060) (Table 4).

Correlation with CAT -262C/T polymorphism (Table 5)

There was no significant difference observed between cases and controls (p=0.711), molecular response (p=0.865) and presence or absence of TKD mutations (p=0.708) with CAT-262C/T polymorphism. This polymorphism was not assciated with risk of CML. Whereas the homozygous CC genotype and C allele frequencies were found to be elevated in the deceased group (71.42%, 0.857) compared to those patients on follow-up (50.0%; 0.711) (p=0.139). The prognostic risk scores were not associated with this polymorphism.

Haplotype analysis of the CAT gene (Table 6)

The haplotype analysis of the *CAT* gene polymorphisms (-21A/T and -262C/T) were performed and represented in Table 6. The haplotypes ATCC and ATCT conferred higher incidence of CML risk by 2.67 times (OR=2.678, 95% CI: 1.051-6.825, p=0.05) and 2.99 times (OR=2.995, 95% CI: 1.116-8.037, p=0.045).

Correlation with GPX1 -198C/T polymorphism (Table 7)

The heterozygous CT genotype and T allele frequencies were significantly increased in CML patients compared to controls (p=<0.0001). With respect to molecular response and TKD mutation status, the heterozygous CT genotype frequency was observed to be significantly elevated in poor molecular responders group (patients having higher *BCR-ABL1* levels) (p=0.005), TKD mutation carriers (p=0.114) and in patients of advanced phases (p=0.292) compared to respective groups. With respect to present status, the frequencies of TT genotype and T alleles were found to be slightly increased in deceased group of patients (23.80%; 0.619) compared to those on follow-up (17.30%; 0.552) (p=0.404). No significant variations were found with prognostic risk scores.

In addition, the *GPX1* -198C/T polymorphism increased the risk of CML under codominant model [CC vs CT (OR=7.316, 95% CI: 3.198-17.736, p=<0.0001), CC vs TT (OR=9.259, 95% CI: 3.489-24.571, p=<0.0001)] and dominant models [CC vs CT+TT (OR=7.628, 95% CI: 3.362-17.310, p=<0.0001)], whereas overdominant model (CT vs CC+TT) was found to be protective against CML (OR=0.432, 95% CI: 0.262-0.711, p=0.001) (Table 8).

Correlation with MPO -463G/A polymorphism (Table 9)

The *MPO* -463G/A polymorphism demonstrated no significant association between cases and controls (p=0.494), nor with either of the confounding variables like molecular response (p=0.465), TKD mutation status (p=0.392), present status (p=0.767) and prognostic risk scores.

Correlation with GSTM1 & GSTT1 null/deletion polymorphism (Table 10)

No significant association observed with *GSTM1* null polymorphism between cases and controls, molecular response, presence or absence of TKD mutations. Whereas *GSTM1* presence genotype (M1) was found to be elevated in deceased group (80.95%) compared to those on follow-up (66.34%) (p=0.289).

With *GSTT1* null polymorphism, the *GSTT1* null genotype frequency slightly increased in cases compared to controls (22.4%; 16.0%) (p=0.193). When the results are stratified with confounding variables, the *GSTT1* null genotype frequency was found to be higher in non-responders (27.05%; 12.5%) and in patients not carrying TKD mutations (26.31%; 10.0%) compared to respective groups. There was no difference was observed between follow-up and deceased group of patients with *GSTT1* null genotype.

No significant differences were found between *GSTM1* & *GSTT1* null/deletion polymorphisms and prognostic

Table 2. Patient Baseline Characteristics (n=125)

	No	%
Gender		
Males	77	61.6
Females	48	38.4
Age at onset		
≤ 42 years	60	48
> 42 years	65	52
Phase		
Chronic	102	81.6
Acute	23	18.4
Sokal risk		
Low	47	37.6
Intermediate	33	26.4
High	45	36
Hasford risk		
Low	49	39.2
Intermediate	49	39.2
High	27	21.6
EUTOS risk		
Low	90	72
High	35	28
BCR-ABL1 levels		
< 10%	40	32
> 10%	85	68
TKD mutations		
Presence	30	24
Absence	95	76
Present status		
Follow-up	104	83.2
Deceased	21	16.8

risk scores.

Haplotype, Linkage Disequilibrium (LD) and Cox Regression analysis:

The haplotype and pairwise epistasis among six SNPs did not revealed any significant association, hence data not presented. The linkage disequilibrium (LD) analysis revealed that the two *CAT* -21A/T (rs1001179) and *CAT* -262 C/T (rs7943316) exhibited high LD (D'=0.9). Since the two SNPs are located on chromosome 1, the observed significant LD might be attributed to the physical proximity. None of the other SNP combinations showed significant LD with D'<0.5 (Figure 1). Cox regression analysis of SNPs with *BCR-ABL1* levels revealed no significant association.

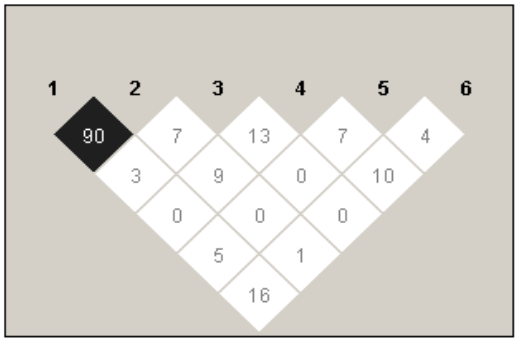

Figure 1. LD plot

Discussion

In the present study, we investigated the association of the genetic variations of the antioxidant enzymes: *CAT* (-21A/T, rs7943316 & -262C/T, rs1001179), *GPX1* (-198C/T, rs1050450), *MPO* (-463G/A, rs2333227) and *GSTM1* (rs366631) & *GSTT1* genes (rs17856199) with susceptibility to CML and their correlation with imatinib (TKI) response.

Our results revealed statistically significant association of *CAT* -21A/T (p=0.037) and *GPX1* -198C/T (p<0.0001) polymorphisms with the risk of CML. No significant associations were observed between *CAT* -262C/T (p=0.711), *MPO* -463G/A (p=0.494), *GSTM1* and *GSTT1* null/deletion polymorphisms (p=1; p=0.193) and CML.

Catalase is an endogenous antioxidant enzyme involved in ROS neutralizing pathways and prevents

Table 3. Genotyping of *CAT* -21A/T Polymorphism

	Genotype frequency				Allele frequency		p value
	AA	AT	TT	Total	A	T	
CML cases	7 (5.6%)	66 (52.8%)	52 (41.6%)	125	0.32	0.68	0.037
Controls	26 (13.0%)	83 (41.5%)	91 (45.5%)	200	0.337	0.662	
BCR-ABL1 levels							
< 10%	4 (10.0%)	22 (55.0%)	14 (35.0%)	40	0.375	0.625	0.259
> 10%	3 (3.52%)	44 (51.76%)	38 (44.70%)	85	0.294	0.705	
TKD mutations							
Presence	2 (6.66%)	18 (60.0%)	10 (33.33%)	30	0.366	0.633	0.571
Absence	5 (5.26%)	48 (50.52%)	42 (44.21%)	95	0.305	0.694	
Present status							
Follow-up	5 (4.80%)	54 (51.92%)	45 (43.26%)	104	0.307	0.692	0.548
Deceased	2 (9.52%)	12 (57.14%)	7 (33.33%)	21	0.38	0.619	

Table 4. Distribution of Odds Ratios between Cases vs Controls with CAT -21A/T Polymorphism

	CML patients (n=125)	Controls (n=200)	OR (95% CI)	p value
Codominant				
AA	7 (5.6%)	26 (13.0%)		
AT	66 (52.8%)	83 (41.5%)	2.953 (1.206-7.228)	0.024
TT	52 (41.6%)	91 (45.5%)	2.122 (0.861-5.228)	0.145
Dominant				
AA	7 (5.44%)	26 (13.0%)		
AT+TT	118 (94.55%)	174 (7.0%)	2.518 (1.058-5.992)	0.050
Recessive				
AT+AA	73 (58.50%)	109 (54.5%)		
TT	52 (41.49%)	91 (45.5%)	0.853 (0.543-1.340)	0.565
Overdominant				
AT	66	83		
AA+TT	59	117	0.632 (0.404-0.994)	0.06

Table 5. Genotyping of *CAT* -262 C/T Polymorphism

	Genotype frequency				Allele frequency		p value
	CC	CT	TT	Total	C	T	
CML cases	67 (53.6%)	50 (40.0%)	8 (6.4%)	125	0.736	0.264	0.711
Controls	116 (58.0%)	71 (35.5%)	13 (6.5%)	200	0.757	0.242	
BCR-ABL1 levels							
< 10%	21 (52.5%)	17 (42.5%)	2 (5.0%)	40	0.737	0.262	0.865
> 10%	46 (54.11%)	33 (38.82%)	6 (7.05%)	85	0.735	0.264	
TKD mutations							
Presence	16 (53.33%)	13 (43.33%)	1 (3.33%)	30	0.75	0.25	0.708
Absence	51 (53.68%)	37 (38.94%)	7 (7.36%)	95	0.731	0.268	
Present status							
Follow-up	52 (50.0%)	44 (42.30%)	8 (7.69%)	104	0.711	0.288	0.139
Deceased	15 (71.42%)	6 (28.57%)	0	21	0.857	0.142	

Table 6. Distribution of *CAT* (-21A/T and -262C/T) Halotypes and their Correlation with Risk of CML

Haplotypes	CML patients (n=125)	Controls (n=200)	OR (95% CI)	p value
AACC	7 (5.6%)	25 (12.5%)		
AACT	0	1 (0.5%)		
AATT	0	0		
ATCC	39 (31.2%)	52 (26.0%)	2.678 (1.051-6.825)	0.05
ATCT	26 (20.8%)	31 (15.5%)	2.995 (1.116-8.037)	0.045
ATTT	1 (0.8%)	0		
TTCC	21 (16.8%)	40 (20.0%)	1.875 (0.696-5.049)	0.31
TTCT	24 (19.2%)	38 (19.0%)	2.556 (0.845-6.019)	0.157
TTTT	7 (5.6%)	13 (6.5%)	1.923 (0.554-0.298)	0.475

cellular injury from ROS [22]. Two polymorphisms: *CAT* -21A/T with altered gene expression pattern [23] and *CAT* -262C/T with lower *CAT* enzyme activity [24] may alter ROS detoxification and increase oxidative stress, implicating oxidative DNA damage and modulating disease risk [25]. In the present study, the *CAT* -21A/T polymorphism was significantly associated with increased risk of CML (p=0.037).

The stratified genotyping results with various confounding variables revealed that the homozygous variant TT genotype increased in non responders (p=0.259) and the heterozygous AT genotype frequency in TKD mutation carriers (p=0.571) and in deceased (p=0.548) group of patients. In addition, the codominant model (AA vs AT) (p=0.024) and dominant models (combined AT and TT genotypes) (p=0.05) presented significant association with increased risk of CML when compared with AA homozygote. Whereas Liu et al (2016)

Table 7. Genotyping of *GPX1* -198C/T Polymorphism

	Genotype frequency				Allele frequency		p value
	CC	CT	TT	Total	C	T	
CML cases	7 (5.6%)	95 (76.0%)	23 (18.4%)	125	0.436	0.564	<0.0001
Controls	62 (31.15%)	115 (57.78%)	22 (11.05%)	199	0.6	0.399	
BCR-ABL1 levels							
< 10%	6 (15.0%)	26 (65.0%)	8 (20.0%)	40	0.475	0.525	0.005
> 10%	1 (1.17%)	69 (81.17%)	15 (17.64%)	85	0.417	0.582	
TKD mutations							
Presence	1 (3.33%)	27 (90.0%)	2 (6.66%)	30	0.483	0.516	0.114
Absence	6 (6.31%)	68 (71.57%)	21 (22.10%)	95	0.421	0.578	
Present status							
Follow-up	7 (6.73%)	79 (75.96%)	18 (17.30%)	104	0.447	0.552	0.404
Deceased	0	16 (76.19%)	5 (23.80%)	21	0.38	0.619	

Table 8. Distribution of Odds Ratios between Cases vs Controls with *GPX1* -198C/T Polymorphism

	Cases	Controls	OR (95% CI)	p value
Codominant				
CC	7 (5.6%)	62 (31.15%)		
CT	95 (76.0%)	115 (57.78%)	7.316 (3.198-17.736)	<0.0001
TT	23 (18.4%)	22 (11.05%)	9.259 (3.489-24.571)	< 0.0001
Dominant				
CC	7 (55.10%)	62 (58.0%)	7.628 (3.362-17.310)	< 0.0001
CT+TT	118 (44.89%)	137 (42.0%)		
Recessive				
CT+CC	102 (93.87%)	177 (93.5%)	1.814 (0.963-3.417)	0.089
TT	23 (6.12%)	22 (6.5%)		
Overdominant				
CT	95	115		
CC+TT	30	84	0.043 (0.262-0.711)	0.001

Table 9. Genotyping of *MPO* -G463A Polymorphism

	Genotype frequency				Allele frequency		p value
	GG	GA	AA	Total	G	A	
CML cases	90 (72.0%)	33 (26.4%)	2 (1.6%)	125	0.852	0.148	0.494
Controls	135 (67.5%)	58 (29.0%)	7 (3.5%)	200	0.82	0.18	
BCR-ABL1 levels							
< 10%	26 (65.0%)	13 (32.5%)	1 (2.5%)	40	0.812	0.187	0.465
> 10%	64 (75.29%)	20 (23.52%)	1 (1.17%)	85	0.87	0.129	
TKD mutations							
Presence	19 (63.33%)	10 (33.33%)	1 (3.33%)	30	0.8	0.2	0.392
Absence	71 (74.73%)	23 (24.21%)	1 (1.05%)	95	0.868	0.131	
Present status							
Follow-up	74 (71.15%)	28 (26.92%)	2 (1.92%)	104	0.846	0.153	0.767
Deceased	16 (76.15%)	5 (23.80%)	0	21	0.88	0.119	

reported an increased cancer risk with recessive model and homozygote model [26]. This indicates that variant T allele with lower catalase activity and thus increased levels of ROS may contribute to genomic instability and increased risk of cancer. Earlier studies reported no significant associations with the risk of colorectal cancer [27], gastric cancer (GC) and hepatocellular carcinoma (HCC) [13].

In our study, we found no evidence of the *CAT* -262 C/T polymorphism with CML risk or its association with confounding variables. Our results are in accordance with earlier studies that reported no significant association with

Table 10. Genotyping of *GSTM1* & *GSTT1* Deletion Polymorphism

	Genotype frequencies						
	T1	T0	p value	M1	M0	p value	Total
CML cases	97 (77.6%)	28 (22.4%)	0.193	86 (68.8%)	39 (31.2%)	1	125
Controls	168 (84.0%)	32 (16.0%)		136 (68.0%)	64 (32.0%)		200
BCR-ABL1 levels							
< 10%	35 (87.5%)	5 (12.5%)	0.111	27 (67.5%)	13 (32.5%)	1	40
> 10%	62 (72.94%)	23 (27.05%)		59 (69.41%)	26 (30.58%)		85
TKD mutations							
Presence	27 (90.0%)	3 (10.0%)	0.105	22 (73.33%)	8 (26.66%)	0.698	30
Absence	70 (73.68%)	25 (26.31%)		64 (67.36%)	31 (32.63%)		95
Present status							
Follow-up	80 (76.92%)	24 (23.07%)	0.92	69 (66.34%)	35 (33.65%)	0.289	104
Deceased	17 (80.95%)	4 (19.04%)		17 (80.95%)	4 (19.04%)		21

risk of hepatocellular carcinoma [28], breast cancer [29], and gastric cancer [30]. Previous other studies showed significant increased risk of cervical cancer [15], breast cancer [31], hepatocellular carcinoma [32] and prostate cancer [33]. Whereas others reported that -262C/T polymorphism was a protective factor with respect to chronic myeloid leukemia [19] and hepatocellular carcinoma susceptibility [14-17].

GPX1 is a key enzyme of the antioxidative system that detoxifies peroxide radicals and lipid hydroperoxides. The -198C/T (Pro200Leu) polymorphism in *GPX1* is associated with reduced enzyme activity [34-35]. Previous studies reported that higher *GPX1* activity is required to counterbalance the ROS levels and related damage occurring during initiation or progression of the cancer [36-39]. We obseved statistically significant association of the homozygous variant TT genotype with CML risk (p=<0.0001). The stratified results of confounding variables presented the significant association of *GPX1* -198 C/T polymorphism with poor molecular response (p=0.005) and acquired TKD mutations (p=0.114). In addition, the codominant (CC vs CT and CC vs TT) and dominant (CC vs CT+TT) models conferred increased risk of CML when compared with CC homozygote (p=<0.0001). Our results were in accordance with others findings on breast cancer [39-41], bladder cancer [42] and lung cancer [43]. This indicates that the variant Leu allele with reduced enzyme activity might increase ROS levels thereby induced oxidative DNA damage and increased susceptibility to cancer. Whereas other studies failed to find an association of *GPX1* -198C/T polymorphism with the risk of CML [19], breast cancer [44-45] and prostate cancer [46].

Glutathione S-transferases (GSTs) are involved in detoxification of a wide range of carcinogens and ROS thereby offering protection against oxidative DNA damage. GST enzymes are polymorphic, which may contribute to the inter-individual variability in the response to oxidative stress suggesting its role in carcinogenesis and risk for cancer. In the present study, the *GSTM1* and *GSTT1* null/deletion polymorphisms were not associated with risk of CML. Our results are similar with earlier studies on CML [20]. Previous studies on the *GSTT1* null polymorphism reported positive association with risk of CML [47-50] and AML [20]. Earlier studies on *GSTM1* null polymorphism showed no association the risk of CML [50], AML [51] and breast cancer [52].

Myeloperoxidase (*MPO*) is an endogenous oxidant enzyme that activates carcinogens [53]. A single nucleotide polymorphism in the promoter region of the *MPO* gene, G-463A (rs2333227) has been associated with reduced mRNA expression and transcriptional activity and subsequent decreased metabolic activation of procarcinogens [54]. In the present study, no evidence of *MPO* -463G/A polymorphism with the risk of CML was observed. Our results were in accordance with earlier studies on ALL [55], AML [56] and breast cancer [57]. Whereas others reported that the A allele with reduced *MPO* activity and ROS production has been associated with decreased risk of breast cancer [58], lung cancer [59] and prostate cancer [60].

In conclusion, our results suggest that the reduced activity of antioxidant enzymes caused by the *CAT*-21A/T and *GPX1*-198C/T polymorphisms might contribute to increased risk of CML. In addition, the *GPX1*-198C/T polymorphism was associated with poor molecular response and acquired TKD mutations. Hence, the present study indicates that defective antioxidant defense system might have a strong influence on CML susceptibility and TKI (imatinib) response through oxidative stress.

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Conflicts of interest

There are no conflicts of interest.

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