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Association of MDR1 gene polymorphism (G2677T) with chronic myeloid leukemia

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Abstract

Imatinib mesylate is the most opted drug used for treating ph+ve chronic myeloid leukemia (CML) patients. The up-regulation of drug transporters (ABCB1-ABCG2) is one of specific causes of Imatinib resistance. Imatinib (IM) is a substrate of the P-glycoprotein pump, which is encoded by MDR1/ABCB1 gene. Our main objective is to investigate the influence of MDR1 gene polymorphisms in CML patients. A total of 262 CML and 252 control samples were analysed for MDR1 gene (G2677T) polymorphism using PCR- RFLP technique. Genotype distribution revealed slight elevation of TT genotype frequency in CML patients (42.7%) compared to controls (38.5%). Patients in advanced phase had higher TT genotype frequency compared to patients in early phases. The frequency of heterozygous GT genotype was found to be increased in hematological poor responders (52.4%) compared to major responders (32.4%) and minor responders (30.0%). Interestingly, the frequencies of GG and GT genotypes were increased in cytogenetic poor responders with corresponding increase in G allele frequency (0.54) as compared to major responders (0.34). These results suggest that the 2677G allele with increased efflux of IM might be responsible for poor response in CML patients.

Keywords: Imatinib; chronic myeloid leukemia; MDR1 gene; polymorphism; drug response.

Introduction

MDR1 codes for P-glycoprotein (P-gp) of 170 KD, an ATP dependent transmembrane transporter that was initially identified by its overexpression in human tumor cells after cancer chemotherapy where it was associated with multidrug resistance (Ueda *et al.,* 1986). It plays a significant role in ADME (absorption, distribution, metabolism, and excretion) processes (Gottesmann *et al.,* 2002) and drug-drug interaction (Callen *et al.,* 1987; Mizuno *et al.,* 2003; Sun *et al.,* 2004). The MDR1 gene is located on the long arm of 7th chromosome at q21.1 and consists of 28 exons which encodes for polypeptide of 1280 amino acids (Chen *et al.,* 1990).

MDR1 gene is highly polymorphic and genetic variants of MDR1 gene are responsible for interindividual variability in pharmacokinetics and dynamics of many drugs. Komar, (2007) reported 50 SNPs in MDR1 gene (Komar *et al.,* 2007), associated with diseases related to pesticide exposure (Drozdzik *et al.,* 2003) and other types of cancers (Potocink *et al.,* 2002; Siegsmund *et al.,* 2002). Mochida, (2003) reported that over expression of P-gp was associated with decreased proliferation activity and slower migration of enterocytes, leading to prolonged life span and resistance to apoptosis resulting in an increased chance of cell transformation (Mochida *et al.,* 2003).

Imatinib mesylate (IM), 2-phenyl aminopyrimidine is the first selective protein tyrosine kinase inhibitor developed for targeted cancer therapy, and is highly effective against several tyrosine kinases such as Abl, kit and the platelet derived growth factor receptor (PDGFR) (Capdeville *et al.,* 2002). It is the most opted drug used for treating ph+ve chronic myeloid leukemia (CML) patients. Imatinib specially targets the tyrosine Kinase domain and blocks the phosphorylation, which is needed for kinase activation and signal transduction. Most of the CML patients achieve major responses, but a proportion of them had acquired resistance. Resistance is more common in patients who start imatinib in late chronic phase and occurs in approximately 70% of patients treated in myeloid blast crisis and in >90% of those in lymphoid blast crisis.

The up-regulation of drug transporters (ABCB1-ABCG2) is one of specific causes of Imatinib resistance, since it can be effluxed through MDR1 (ABCB1) transporters (Gurney *et al.,* 2007). Upregulation of drug transporters results in enhanced clearance of drug from the cell resulting in reduced drug availability and drug resistance. Hence, the study of G2677T polymorphism in exon 21 of MDR1 gene will be helpful in dose adjustments of imatinib, which should be effective in case of Imatinib resistant individuals.

Materials and Methods

The present study includes 262 CML and 252 control samples. Blood samples were collected from CML patients being treated at NIMS (Nizam's Institute of Medical Sciences), Hyderabad reported during 2004-2006. The age and sex matched control samples without history of cancer were randomly selected from different localities in Andhra Pradesh. Patients clinical data like phase of the CML and response (Hematological and Cytogenetic) to therapy was noted from the tumor registry file with the help of oncologist. Response status (hematological and cytogenetic) was classified on the basis of the total leukocyte count, percentage of ph+ve cells and duration of response to imatinib therapy (Druker et al., 2005). Genomic DNA was isolated by using salting-out method (Lahiri *et al.,* 1991) and used for polymorphic analysis using PCR-RFLP technique.

Analysis of G2677T polymorphism

The G2677T polymorphism was analysed using a set of primers F5'- TGC AGG CTA TAG GTT CCA GG – 3' and R5'- TTT AGT TTG ACT CAC CTT CCC G – 3' to amplify 224bp fragment. The PCR cycling conditions include initial denaturation at 94^0C for 5 minutes followed by 35 cycles of denaturation at 94^0C for 30 seconds, annealing at 58^0C for 30 seconds, extension at 72^0C for 45 seconds and final extension at 72^0C for 10 minutes. The amplified PCR products were digested with Ban I restriction enzyme (New England Biolabs) for 1 hour at 37°C. After digestion, the products were electrophoresed on a 2% agarose gel for genotyping. 2677G allele creates site for Ban I enzyme and produces two fragments of 198 bp and 26 bp whereas 2677T allele was identified by single fragment of 224 bp (figure 1).

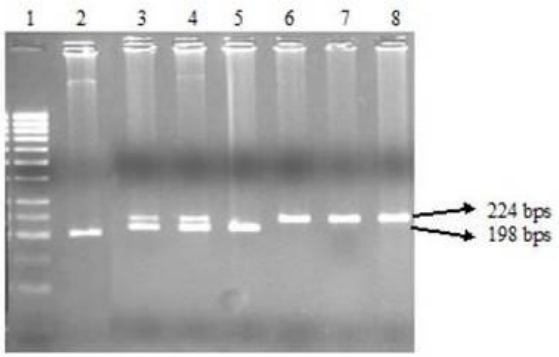
Statistical analysis

All the statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) 15.0. Chi square test was 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 $p<0.05$.

Results

The frequency distribution of MDR1 gene (G2677T) polymorphism was represented in table 1. In the present study, the frequency of 2677 TT genotype was found to be increased slightly in CML patients (42.7%) compared to controls (38.5%). No significant association of G2677T polymorphism was observed with respect to age at onset and sex of the proband. When the association of G2677T polymorphisms with clinical phase was considered, heterozygous 2677GT genotype frequency was increased in patients with blast crisis (45.55%) compared to patients in accelerated (26.3%) and chronic phase (31.8%). With respect to drug response status, the frequency of heterozygous 2677 GT genotype was increased in hematological poor responders (52.4%) compared to major responders (32.4%) and minor responders (30.0%). Interestingly, the frequencies of 2677GG and GT genotypes were increased in cytogenetic poor responders with corresponding increase in G allele frequency (0.54) as compared to major responders (0.34) (Table 1).

Figure 1. MDR1 (G2677T) Polymorphism



Lane 1 – 50bp ladder
Lanes 2, 5 – GG (Homozygous)
Lanes 3, 4 – GT (Heterozygous)
Lanes 6, 7, 8 – TT (Homozygous)

Table 1. Distribution of MDR1 G2677T polymorphism in Chronic Myeloid Leukemia with respect to epidemiological and clinical parameters.

Parameters	GG	GT	TT	Allele frequency	
	No %	No %	No %	G	T
Cases (262)	64 (24.4)	86 (32.8)	112 (42.7)	0.408	0.591
Controls (252)	83 (32.9)	72 (28.6)	97 (38.5)	0.472	0.527
		χ 2= 4.58; df = 2, p = 0.101			
Age at Onset					
< 20 Yrs (25)	7 (28.0)	8 (32.0)	10 (40.0)	0.44	0.56
20-30 Yrs (67)	14 (20.9)	18 (26.9)	35 (52.2)	0.343	0.656
30-40 Yrs (61)	15 (24.6)	23 (37.7)	23 (37.7)	0.434	0.565
> 40 Yrs (109)	28 (25.7)	37 (33.9)	44 (40.4)	0.426	0.573
		χ 2= 3.72; df = 6, p = 0.714			
Sex of the Proband					
Males (180)	42 (23.3)	60 (33.3)	78 (43.3)	0.4	0.6
Females (82)	22 (26.8)	26 (31.7)	34 (41.5)	0.426	0.573
		χ 2= 0.37; df = 2, p = 0.831			
Phase of CML					
Chronic (214)	51 (23.8)	68 (31.8)	95 (44.4)	0.397	0.602
Accelerated (19)	7 (36.8)	5 (26.3)	7 (36.8)	0.5	0.5
Blast Crisis (22)	5 (22.7)	10 (45.5)	7 (31.8)	0.454	0.545
		χ 2= 3.53; df = 4, p = 0.473			
Hematological Response					
Major (185)	44 (23.8)	60 (32.4)	81 (43.8)	0.4	0.6
Minor (10)	2 (20.0)	3 (30.0)	5 (50.0)	0.35	0.65
Poor (21)	2 (9.5)	11 (52.4)	8 (38.1)	0.36	0.64
		χ 2= 4.24; df = 4, p = 0.374			
Cytogenetic Response					
Major (136)	24 (17.6)	44 (32.4)	68 (50.0)	0.34	0.66
Minor (29)	7 (24.1)	11 (37.9)	11 (37.9)	0.43	0.57
Poor (41)	13 (31.7)	18 (43.9)	10 (24.4)	0.54*	0.46
		χ 2= 9.28; df = 4, p = 0.0545*			

Discussion

The SNP G2677T in exon 21 (893 codon), results in the substitution of alanine to serine/threonine in such a manner that the lipophilic residue (Ala) is changed to hydrophilic residue (Ser, Thr) conferring higher resistance to various drugs such asadriamycin and vinblastine (Kioka *et al.,* 1989; Kim *et al.,* 2001). The G2677T polymorphism was significantly associated with increased or decreased plasma concentration of P-gp substrates (Kurata *et al.,* 2002; Siegmund *et al.,* 2002). Recent reports showed that individuals who had the 2677 TT genotype had lower P-gp messenger RNA expression than those who had 2677 GG genotype (Lamba *et al.,* 2006). On the contrary, some pharmacokinetic studies reported an opposite effect of the 2677T mutant allele, i.e. an increase in transport activity (Kurata *et al.,* 2002) compared with that of 2677G allele, whereas Tanabe (2001) reported a non-significant opposite trend for P-gp expression in placenta in relation to the G2677T polymorphism (Tanabe *et al.,* 2001). These contradictions might be due to the presence of different amino acids at position 893, which might have different effects on different drugs.

In our study, the frequency of TT genotype was increased slightly in CML patients compared to controls. Although the results were found to be insignificant, 2677TT genotype with lower P-gp expression might confer risk to develop CML due to decreased ability to transport environmental carcinogens as reported in lung cancer study (Gervasini *et al.,* 2006).

Heterozygous 2677GT frequency was increased in hematological poor responders, while the frequencies of GG genotype and 2677G allele were elevated in cytogenetic poor responders. This indicates that 2677G allele carriers might be at risk for drug resistance. The G allele was reported to have enhanced P-gp expression and was observed to be associated with increased efflux of P-gp substrates. Since imatinib is one of the P-gp substrate, the present results indicated that G allele was associated with poor response to imatinib in CML patients. Previous study by Dulucq (2008) reported that the G allele at 2677 position was significantly associated with worse response in chronic myeloid leukemia patients who were on Imatinib therapy (Dulucq *et al.,* 2008). Another study on AML patients reported that 2677T allele was associated significantly with shorter relapse time and survival rates compared to heterozygotes (Van den *et al.,* 2001). In some other studies, haplotype of 2677TT and 3435TT was associated with highest risk of drug resistance in lymphoproliferative diseases (Goreva *et al.,* 2004).

Conclusion

In the present study, the G allele at 2677 position was significantly associated with worse response in chronic myeloid leukemia patients who were treated with Imatinib, as Imatinib was one of the substrate of the P-glycoprotein pump. Hence, genotyping of MDR1 gene polymorphism (G2677T) might be helpful in planning the individualized therapy based on the genotypes.

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