Frequency Distribution of CYP2C19 Gene Polymorphism in Acute lymphoblastic leukemia (ALL) Patients in Punjab

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Abstract- Cytochrome P450 (CYP) 2C19 is a phase I enzyme that is involved in the metabolism of xenobiotics, drugs including clopidogrel, cyclophosphamide, lansoprazole, and diazepam, as well as carcinogens, pesticides, and food toxins. Inter-individual differences in metabolizing exogenous and endogenous compounds are caused by genetic polymorphisms in the CYP2C19 gene, which encodes the enzyme, and is also linked to the risk of various diseases. A mutation in the CYP2C19 gene was expected to increase the risk of Lymphoblastic leukemia (ALL). However, there is no comparable data on the genetic variation of the CYP2C19 gene in ALL Punjabi patients. The genotyping of CYP2C19*2 polymorphism was performed in 50 ALL patients and 50 age and gender-matched controls from Punjab, India by using the PCR-RFLP method in order to investigate the frequency and influence of CYP2C19*2 polymorphism in susceptibility to ALL. It is indicated that the genotype distribution of CYP2C19*2 polymorphism in cases and controls was significantly different (P=0.03*). The GA genotype of CYP2C19*2 polymorphism may be associated with increased risk to ALL with OR= 3.7: 95% CI: 0.93-14.6: P-value =0.04*. It was concluded that CYP2C19 * 2 polymorphism is found to be associated with ALL.

Keywords:- CYP2C19, ALL, Xenobiotics, Gene polymorphism, SNPs.

1. Introduction

Xenobiotics are extracellular chemicals that are not synthesized by the organism. Humans are continually exposed to xenobiotics, which induce genetic mutations that lead to the development of leukemia. Toxicity is induced by xenobiotics in the absence of metabolism . To metabolize these xenobiotics, the human body has an enzymatic metabolic system. There are three types of xenobiotic-metabolizing enzymes: phase I, phase II, and transporter enzymes. The cytochrome P450 (CYPs) enzymes are important players in phase I metabolism [1], and they play a role in the metabolism of carcinogens and drugs like omeprazole, lansoprazole, proguanil, and cyclophosphamide. [2–3]. The CYP2C19 gene is strongly polymorphic and influences the metabolism of a wide variety of therapeutic drugs. The CYP2C19 gene is located on chromosome 10q 23.33 and contains 9 exons and 8 introns [4]. It has a 1473 bp coding sequence that results in a protein with 490 amino acid residues. There are approximately 25 genetic variants that have been identified in the exonic region of the CYP2C19 gene. CYP2C19*2, CYP2C19*3, and CYP2C19*17 are

common CYP2C19 gene variants associated with drug metabolism. CYP2C19*2 and CYP2C19*3 are most often found in people who have a diminished capacity to metabolize drugs. Furthermore, the

CYP2C19*17 variant is linked to ultra-rapid metabolism. The CYP2C19*2 allele is caused by a guanine (G) to adenine (A) transition at position 681 in exon 5, which results in an irregular splice. CYP2C19*3 is a significant allele in which a G to A transformation occurs at position 636 in exon 4 of the CYP2C19 gene, resulting in an early stop codon and truncated protein[7-8]. There is currently no reliable data on the CYP2C19*2 polymorphism in the Punjab population. The main goal of this research is to determine the allele frequency of CYP2C19*2 in the Punjabi population and to equate the results to those of other studies conducted around the world.

1. MATERIAL AND METHODS

1.1 Subjects

A total of 50 patients with acute lymphoblastic leukemia (ALL) and 50 age and gender matched healthy controls (N=50) were included in the case control study. After pathological confirmation at the Sandhu Cancer Centre in Ludhiana, the patients were selected. The control group consisted of individuals of similar age and gender who had no personal or family history of cancer and were recruited from various blood donation camps. Peripheral blood samples were obtained in EDTA-coated vials. The Institutional Ethical Research Committee approved the report, and all cases and controls gave written informed consent.

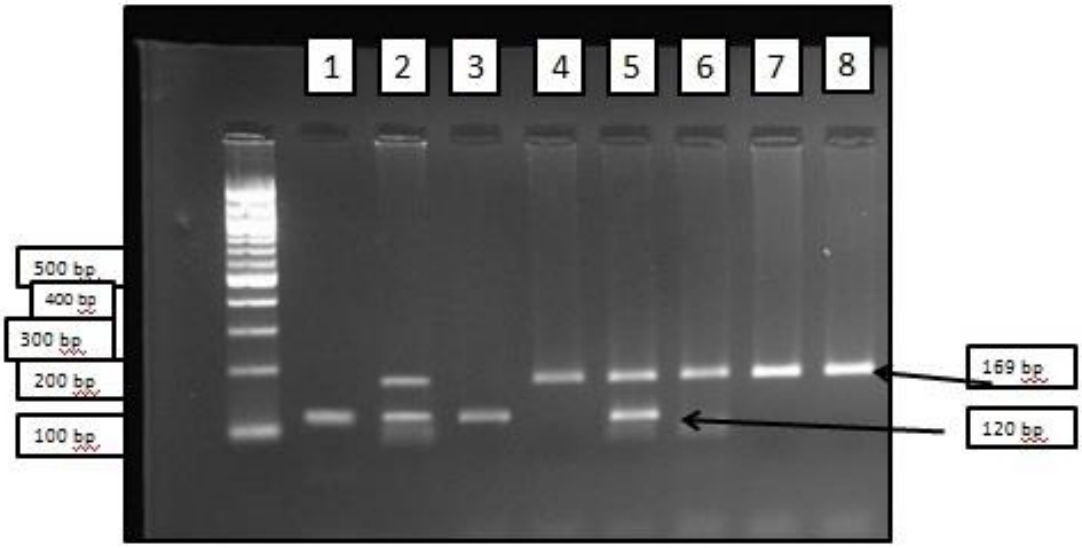
1.2 Extraction of DNA

Genomic DNA was extracted from blood samples using an inorganic (salting out) method given by Miller et al., 1988 [7] and stored at -20°C in a deep freezer until required. A spectrophotometer was used to verify the quality and quantity of DNA.

2.3 CYP2C19*2 genotyping

The CYP2C19*2 genotype was performed by polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP) as described by Zand et al.(2005) and De Morais et al. (1994a,b). The 169 bp PCR product was amplified using the primer pair 5'-AATTACAACCAGAGCTTGGC-3' (Forward) and 5'-TATCACTTTCCATAAAAGCAAG-3' (Reverse) (Table 1). After an initial denaturation at 95^{o}C for 5 min, amplification was carried out for 30 cycles at 94^{o}C for 30 sec, 56^{o}C for 30 sec and 72^{o}C for 30 sec, followed by final extension at 72^{o}C for 5 min. The PCR product (169bp) was then digested with SmaI (NEB) 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. The homozygous wild-type allele (GG) was identified by the presence of 120 bp and 49bp whereas the homozygous variant allele (AA) was confirmed by the presence of fragments of 169 bp size. The heterozygous variant allele (GA) was identified by the presence of 169 bp, 120 bp fragments (**Figure 1**).

Figure 1: Gel electrophoresis showing CYP2C19*2 (rs4244285; 681G>A gene polymorphism by PCR-RFLP



Lanes 1 and 3 represent homozygous wild type alleles (wt/wt) and Lane 2 and 5 represents heterozygous mutant alleles (wt/mt) and Lanes2 and 4, 6, 7 and 8 represent homozygous mutant (mt/mt) genotypes.

Table 1:- Primer sequences an restriction enzyme used

Gene Variation	Gene Variation	Fragment length (bp)	RE	Digestion pattern(bp)		References
				Wild	Variant	
CYP2C19*2 (rs4244285; 681G>A	Forward 5'-AATTACAACCAGAGCTTGGC-3' Reverse 5'-TATCACTTTCCATAAAAGCAAG-3'	169	SmaI	120,49	169	Zand et al,2006 [8]

RE – restriction enzymes

2.4 Statistical analysis:-

A chi-square test was used to compare the variations in genotype frequencies between ALL patients and controls. The odds ratio (OR) at 95 percent confidence limits was used to determine the relationship between ALL risk and CYP2C19*2 polymorphism. The significance level for the analysis was set at $p<0.05$.

3.Results

3.1 Baseline data:

General characteristics of ALL patients and controls are shown in Table 2. Out of 50 ALL patients almost equal number of age and gender matched individuals of respective subtypes of leukemia were taken as controls. Among patients, 19 were females and 31 were males with an average age of 28.4±12.4 years. Among controls, 17 were females and 33 were males with an average age of 30.22±11.11 years. The number of ALL patients were higher in rural areas (62%) as compared to urban areas (38%). The frequency of physically active individuals was lower in ALL patients as compared to controls. Dietary habits, smoking and alcohol drinking showed non-significant differences between cases and controls in overall as well as in different subtypes of leukemia.

Table 2 Baseline characteristics of ALL patients and controls

Variables		**ALL (N=50) n (%)**	**Controls (N=50) n (%)**
Mean age ± SD (Range)		28.4±12.4 (19-28)	30.22±11.11 (20-30)
Gender	Male	31 (62)	33 (66)
	Female	19(38)	17 (34)
Dwelling	Urban	19 (38)	34 (68)
	Rural	31 (62)	16 (32)
Physical activity	Active	18 (36)	42 (84)
	Sedentary	32 (64)	8 (16)
Dietary habits	Veg	31 (62)	27 (54)
	Non-veg	19 (38)	23 (46)
Smoking	Yes	3 (6)	02 (4)
	No	47 (94)	48 (96)
Alcohol drinking	Yes	9 (18)	07 (14)
	No	41 (82)	43 (86)

3.2 RFLP Result and Genotype Frequencies of CYP2C19*2 polymorphism:

The genotypic frequency of CYP2C19*2 polymorphism among ALL patients and controls is given in **Table 3**. A significant difference was observed in genotype distribution among ALL patients and controls (p= 0.03*). High percentage of GA (18%) genotype was found in ALL patients as compared to GA (6%) genotype of controls. The frequency of the homozygous mutant genotype(AA) of CYP2C19*2 was found to

be 6% in the ALL patients and no homozygous mutant genotype was found in controls(Table 3).

Odds ratio (OR) with 95% confidence intervals (CI), were calculated for each group to estimate the association between the CYP2C19*2 polymorphism and the risk of ALL in the population of Punjab (Table 4). It was found that CYP2C19*2 GA genotype was associated with 3.7-fold (OR=3.7, 95% CI, 0.93- 14.6; p= 0.04*) higher risk. While 'A' allele showed association with 5.7-fold increased susceptibility with AL (OR = 5.7, 95% CI, 1.5- 20.3; p =0.003*). Genetic model analysis revealed higher risk for ALL only under the dominant model (OR=4.9 ; 95% CI=1.3- 18.80).

Table 3. Genotype frequency of CYP2C19*2 polymorphism among ALL patients and controls

Genotype	GG	GA	AA	Chi -square	p-value
ALL Patients	38(76%)	9(18%)	3(6%)	6.9	0.03*
Controls	47(94%)	3(6%)	0(0%)		

Table 4. Association of CYP2C19*2 (rs4244285; 681G>A gene polymorphism with Acute lymphoblastic leukemia (ALL)

Models	**Genotype/ Allele**	**ALL N=50 n (%)**	**Controls N=50 n (%)**	**OR (95%CI)**
Co- dominant	GG	38 (76)	47 (94)	
	GA	9 (18)	3 (6)	3.7 (0.93- 14.6) P= 0.04*
	AA	3 (6)	0 (0)	0.11 (0.005-2.3) P= 0.16
Allele	G	85 (85)	97(97)	
	A	15 (15)	3(3)	5.7 (1.5- 20.3) P= 0.003*
Dominant	GG	38 (76)	47 (94)	
	GA+AA	12 (24)	3 (6)	4.9 (1.3- 18.80) P= 0.01*
Recessive	GG+GA	47(94)	50 (100)	
	AA	3 (6)	0 (0)	0.13 0.006- 2.67) P= 0.18

P<0.05(Statistically significant)

4. Discussion

Exogenous carcinogens and various drugs are metabolised by Phase I and Phase II enzymes, and Cytochrome P450 is a superfamily of Phase I detoxification systems located mainly in the liver. CYPs are a type of enzyme system that aids in the biotransformation of chemicals, toxins, foods, and drugs. CYP2C19 is an enzyme in the CYP family that is involved in the metabolism of nearly 20% of all drugs. Clopidogrel, Lansoprazole, Diazepam, Barbiturate, Nelfinavir, Omeprazole, Cyclophosphamide, and Clonazepam are only a few examples. Variations in drug response are caused by CYP2C19 polymorphism. Aside from their traditional function in metabolism, CYPs are now being investigated for their role in cancer susceptibility and therapeutic outcome, as well as, more recently, drug discovery [16]. According to the literature, there are 27 CYP2C19 mutant alleles, the most common of which are CYP2C19*2 and CYP2C19*3.

The CYP2C19*2 polymorphism has been estimated and compared to global studies.This study reveals the prevalence of CYP2C19*2 allele is 15% in ALL and 2.43% in controls and the combined frequency of CYP2C19*2 allele is 8.98%. The CYP2C19*2 allele was observed with a frequency of 14.4%, 15%, 13.6% and 13.1% among Swedish (10), German (11), Ethiopian (12) and Zimbabwean (13) respectively which is noticed to be less than Iranian population (14) having 21.4%. Moreover, the CYP2C19*2 allele occurred with a frequency of 30% and 36.8% among Chandigarh(15) and South Indian kerala population (16), respectively.

5.Conclusion

It is concluded that CYP2C19*2 has been linked to an increased risk of ALL. More research with a larger sample size is needed to better assess the position in ALL risk. Furthermore, this research may aid pharmacogenetic testing in cancer patients, allowing for a greater role in personalised medicine.

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6. References

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