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Frequency of CYP1A1*2A Polymorphism in Syrian Children with Acute Lymphoblastic Leukemia

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ABSTRACT:

Although acute lymphoblastic leukemia (ALL) is the most common childhood cancer; factors governing susceptibility to this disease have not yet been identified. CYP1A1, a member of the cytochrome P450 (CYP) enzymes, plays a very important role in the metabolism of carcinogens. To explore the contribution of CYP1A1*2A polymorphism to ALL susceptibility, we conducted a case–control analysis in Syrian children. 70 children with acute lymphoblastic leukemia and 45 healthy control were studied. Genomic DNA was extracted, and restriction fragment length polymorphism (RFLP) based PCR was applied followed by digestion with MspI. Among 70 ALL patients, 8.6% were heterozygous for the CYP1A1*2A genotype compared with 4.4% of controls. The differences between the groups were found not to be statistically significant (OR 2.016; 95% CI 0.39-10.46). The results did not show any association between CYP1A1*2A genotypes and risk of ALL in Syrian children.

KEYWORDS: Cytochrome ,CYP1A1*2A, polymorphism, ALL, susceptibility, PCR- RFLP, Syrian children .

INTRODUCTION:

Acute lymphoblastic leukemia (ALL), a malignant disorder of lymphoid progenitor cells, affects both children and adults with peak prevalence between the ages of 2 and 5 years. Steady progress in development of effective treatments has led to a cure rate of more than 80% in children[1]. The etiology of ALL continues to be incompletely explained with few established environmental risk factors[2-4]. Previous reports of childhood cancer have suggested that genetic variants within xenobiotic metabolizing enzymes (XMEs) significantly affect susceptibility to childhood ALL [5-8].

Cytochrome p450enzymes involved in the bioactivation of several chemical carcinogens including environmental carcinogens and reactive oxygen species. Cytochrome enzymes transfer electrons onto toxicants to create highly reactive intermediates which are usually coupled to glutathione or other groups producing water-soluble compounds, but can also interact with DNA, resulting in the formation of DNA adducts[9]. Cytochrome p450 1A1 (CYP1A1), a member of the CYP1 gene family, has the polymorphism" *2A"which is a T6235C change within the 3' noncoding region of the gene. The *2A allele has been associated with higher induction of CYP1A1[10]. This polymorphism determines three genotypes, wt/wt, which is the wild type lacking the MspI cleavage site, wt/m1and m1/m1, which are heterozygous and homozygous respectively for the polymorphic allele with the MspI site[11-12]. A study among Indian children found that CYP1A1 polymorphism greatly increased susceptibility of ALL with the homozygous CYP1A1*2A conferring a 6-fold risk[13].

In Another study among French–Canadian children, polymorphism has proved to be significantly associated with both an increased genetic susceptibility 8 and worse prognosis[14]. The frequency of the CYP1A1*2A polymorphism varies between ethnic groups, but there are no reports describing the frequency or associations of the CYP1A1*2 Apolymorphism with the susceptibility of ALL in patients in the Syrian population. The aim of this study is to evaluate the role of CYP1A1 polymorphism in development of ALL in Syrian children.

MATERIALS AND METHODS:

Subjects:

The case group of the study consisted of (70) consecutive patients with childhood ALL admitted to the Damascus University Children's Hospital. The patients with ALLcomprised47 males (67.14%)and 23 females (32.85%) between the ages of 1.5 months and13 years (mean age 4.4 ± 2.8 years). The distribution of ALL subtypes as determined by flow cytometric analysis was as follows: 59 B-lineage ALL (39 M/20 F), 9 with-lineage ALL (7 M/2 F), and 2 with undetermined lineage. The control group consisted of 45 randomly selected and unrelated patients visiting the same hospital for emergency room without any evidence of malignancy. 26 males (57.8%) and 19 females (42.2%) informed consent was obtained from all participating individuals parents.

Genotyping:

Genomic DNA was extracted from peripheral blood using DNA isolatin kit(Thermo Fisher Scientific Inc)CYP1A1 mutation T6235C (m1), were characterized by the PCR-RFLP[15], a DNA fragment of 340bp was amplified in50µL containing 20 ng of genomic DNA, 0.1µmol/L of primers M1F(5'TAG GAG TCT TGT CTC ATG CCT3') and M1R (5'CAG TGA AGA GGT GTA GCC GCT3'),and 25 µL PCR master mix (Thermo Fisher Scientific Inc). PCR was performed for initial melting step of 5 minutes and 30 cycles of 1 minute at 94°C, 1 minute at 61°C, and 1 minute at 72°C,and a final elongation step of 10 minutes at 72°C. The PCR product (10 µL) was digested with 3 U of MspI (Vivantis)for 3 hr at 37°C resulting in smaller fragments (200and 140 bp) in case of the mutation. Genotypes were analyzed by electrophoresis on a 3% agarosegel containing ethidium bromide.

Statistical Analysis:

Statistical significance of the differences in the frequency of genotypes was assessed using chi square test, and odds ratios (ORs) were calculated along with their 95% confidence intervals (CI). The possible interactions of the genotype distributions with respect to the age, sex, white blood cell count, and immunopheno type groups of ALL patients were assessed by using the chi-square test. The analyses were performed by using the SPSS statistical package (version 16.0).

RESULTS:

Among 70 ALL patients, 8.6% were heterozygous for the CYP1A1*2A genotype, compared with 4.4% of controls. The differences between the groups were found not to be statistically significant (OR 2.016; 95% CI 0.39-10.46).The distributions of CYP1A1 genotypes in ALL patients and controls and the frequencies of risk-elevating allele are given in Table I.

ALL patients were grouped according to immunophenotype, sex, age at diagnosis, and WBC count at diagnosis, and the distributions of the genotypes within each groups are given in Table II.

The frequency of the heterozygous CYP1A1*2A genotype was 8.4% in the group of B-cell ALL as compared to 4.4% of controls and as compared to 11.1% of T-cell ALL. The heterozygous genotype was more frequent in females (17.3%) than in males (4.2%) and in patients with WBC < 10,000 (3.7%) than in patients with WBC > 10,000/mm^3 (16.6%). A statistically significant difference was observed in the distribution of this genotype among the sex groups of all patients (P_0.006).A statistically significant difference was observed in the distribution of this genotype among the WBC count at diagnosis groups of all patients (P_0.008).

TABLE I. Distribution of CYP1A1 Genotypes in ALL Patients and Controls

Genotype	Patients			Controls			p-value	OR	95%CI	
	Total	No.	(%)	Total	No.	(%)				
	70			45						
Non*2A/Non*2A		64	91.4		43	95.6		1.000		
Non*2A/*2A		6	8.6		2	4.4	0.396	2.016	0.39	10.46
*2A/*2A		-								
*2A/*2A, Non*2A/*2A		6	8.6		2	4.4	0.396	2.016	0.39	10.46
*2A allele	140	6	4.3	90	2	2.2	0.405	1.970	0.39	9.98

The statistical significance of the differences between allele frequencies was estimated by chi-square test.

TABLE II. Frequencies of CYP1A1 Genotypes in Groups of ALL Patients

	Total	Non*2A/*2A	P value
Immunophenotype			0.882
pre B ALL	59	5(8.4%)	
pre T ALL	9	1(11.1%)	
undetermined	2		
sex			0.006
male	47	2(4.2%)	
female	23	4(17.3%)	
age group			0.341
0,2	3	1(3.3%)	
2,6	49	4(8.1%)	
6,13	18	1(5.5%)	
WBC/mm3			0.008
≤10000	27	1(3.7%)	
10000;50000	30	5(16.6%)	
≥50000	13	0	

Statistical analysis in each group was performed by chi-square test, and *P* values are given in the table.

DISCUSSION:

The effect of genes that influence susceptibility to leukemia is not clear although the clinical and pathological aspects of this complex disease are well understood[16]. Biotransformation of xenobiotics by phase I and phase II enzymes is an important process in initiating chemical carcinogenesis[17]. The expression of CYP1 family enzymes is increased in lymphoid and myeloblastic cell lines, so CYP1 enzymes may donate to the carcinogenesis of hematopoietic cells[18]. Transformation of the procarcinogens entering the cell into active carcinogens is made by the CYP1A1 enzyme. The difference in activity of this enzyme causes accumulation of DNA adducts in cells. Different kinds of mutations in tumor suppressor genes and oncogenes are caused by Increasing DNA adducts ,so it may trigger cancer cell development. Therefore, people with a distorted ability to activate procarcinogens may have an increased risk of developing cancer.[19] A higher level of DNA adduct formation and increased risk of carcinogenesis is associated withCYP1A1*2A variant due to increased activity[20-23]. CYP1A1*2A appears to be one of the significant risk determinants of ALL according to the study of Sinnett et al[7]. in Another study by Joseph and colleagues, Indian children with homozygous CYP1A1*2A variant allele had an increased risk of childhood ALL conferring a 6-fold risk[13]. Whereas several studies found that CYP1A1*2A variant allele was not related with childhood acute leukemias in Brazilian and Turkish groups[6,24,25]. However, our study results shows no correlation between heterozygous variant allele and a risk of childhood leukemia (OR 2.016; 95% CI 0.39-10.46). These variable data in different populations show that the study of each gene polymorphism is essential for each population. We think there are many gene polymorphisms that play a significant role in leukemia pathogenesis which can help uncover the etiology of leukemia for better diagnosis and treatment. Therefore, a wide-ranging study with a larger sample size is necessary to look at all the suspected genes in order to accomplish the most accurate results.

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