Polymorphism in Cytochrome 45 (*cyp3A5*) and Sulfertranseferase *(SULT1A1*) Genes in Patients with Leukemia

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Abstract

Susceptibility to leukemia can be highly influenced by genetic polymorphisms in metabolizing enzyme genes of environmental carcinogens. This study aimed to evaluate the impact of the *CYP3A5* and *SULT1A1*metabolizing enzyme gene polymorphisms on the risk of leukemia. The analysis was conducted on 100 patients with leukemia referred to Radiation and Isotopes Centre Khartoum *(*RICK), study include 62 male and 38 females patients with leukemia and 100 healthy individuals as control group. patients were fully informed about the aims of the study, besides a approved written consent. Distribution of leukemia among patients revealed that 25% choric myeloid leukemia (CML), 26% chronic lymphatic leukemia (CLL), 21% acute myeloid leukemia (AML) and 28% with acute lymphoblastic leukemia. According to gender occurrence of leukemia in males was 62(62%) with frequencies CML 16 (64%), CLL 14(54%), AML 10 (48%) and ALL 22(79%). The frequency of leukemia among female patients was 38(38%) distributed as CML 9 (36%), CLL 12(46%), AML 11 (52%) and ALL 6(21%). DNA extracted from EDTA blood samples and PCR-RFLP performed for each patient and control subject. The mutant *CYP3A5*3/*3* genotype was more frequent among study group (P value = 0.001) in contrast sulotransferase 1A1 mutant SULT *2/*2 genotype shows insignificant value (P value = 0.446), in relation to type of leukemia CYP3A5*3/*3 frequencies in CML was 11(42.3%), CLL 8(30.8%) AML 3 (11.5%) and ALL 4 (15.4%). While the mutant SULT *2/*2 frequency was 5(45.46%) in CML and 2(18.18%) in CLL, AML and ALL respectively.

Keywords: CYP3A5 gene - SULT1A1gene - leukemia - polymorphism - PCR-RFLP

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Introduction

Genetic variations are thought to be the important factors in the development of this leukemia. Molecular epidemiological studies have proved that genetic polymorphisms of metabolic enzymes influence the risk of a variety of tumors including leukemia.[1] Functional polymorphisms in the genes encoding xenobiotic-metabolizing enzymes cause inter individual differences that contribute to leukemia susceptibility. Cytochrome P450, which belongs to phaseI biotransformation enzymes, is responsible for the metabolism of endogenous as well as exogenous DNA-reactive chemical compounds and xenobiotics which might induce genotoxicity and increase the risk for leukemia[2].

Cytochrome P450 enzymes are the most important enzymes in Phase I metabolism in mammals, and are primarily responsible for the metabolism (degradation and elimination) of drugs, so it can be effectively eliminated by the kidneys. These reactions usually involve either adding or unmasking a hydroxyl group, or some other hydrophilic group such as an amine or sulphydryl group, and usually involve hydrolysis, oxidation or reduction mechanisms. Hence Cytochrome P450 enzymes are responsible for most phase I reactions. [3]

Also the efficacy and toxicity of the drugs can be enhanced in a person by focusing on the phase I and phase II drug metabolism genes e.g. cytochrome P450 family. [4] There are four genes in the CYP3A family.[5] In contrast a huge number of investigations were carried out to find the consequence of genetic variation of CYP3A4 and CYP3A5 both are most commonly involved in drug related reactions. [6] Studies reported that CYP3A4 and CYP3A5 accounts for 36% of activity of all CYP3A genes. CYP3A5*3 (CYP3A5 6986A>G) variant codes a different spliced mRNA with a premature terminator codon, wild type CYP3A5*1 mRNA is more stable than CYP3A5*3 mRNA which is more unstable and quickly degraded. [7]

Meta-analyses indicate that the deletion of GSTM1 and GSTT1 is associated with a slightly increased risk of lung cancer and acute leukemia.[8] Sulfotransferase IA1 plays an important role in the detoxification and hydroxylated metabolites of aromatic amines. SULT1A1 is involved in the metabolism of genotoxic metabolites of 3-nitrobenzanthrone, one of the carcinogenic compounds found in diesel exhaust. [9] Transferase enzymes such as Sulfotransferase 1A1 are responsible for most phase II reactions, and takes place if phase I is insufficient to clear a compound from circulation, or if phase I generates a reactive metabolite. These reactions usually involve adding a large polar group (conjugation reaction), such as glucuronide, to further increase the compound's solubility. Sulfotransferase 1A1 is an enzyme that in humans is encoded by the SULT1A1 gene.[10] Some studies have shown that genetic polymorphism in SULT1A1 gene leads to a decrease in enzymatic activity of SULT1A1 and the sulfonation efficiency thus associating with susceptibility to several cancers. specific role of SULT1A1 Arg213His polymorphism in carcinogenesis was documented. [11]

Sulfotransferase (SULT) enzymes catalyze the sulfate conjugation of a broad range of substrates and play an important role in metabolism of endogenous and exogenous compounds including thyroid and steroid hormones, neurotransmitters, drugs and procarcinogens.[12] There are many isoforms of the *SULT*s supergene family, each with different amino acid sequence identity and substrate specificity [13] SULT1A1 is an important member of the sulfotransferase family involving in the pathogenic process of various cancers and leukemia. [13]

Materials and Methods

A cross sectional hospital based study was conducted in Radiation and Isotopes Centre in Khartoum (RICK) in period 2012 to 2015. A total of 100 patients with leukemia were enrolled and one hundred healthy individuals without leukemia or family history of leukemia or any others cancers were included as control group. Ethical approval was obtained from Ethical Committee (RICK) and informed consent was obtained from the patients or their parents. DNA extracted from EDTA blood samples and stored at-20°C for PCR-RFLP. CYP3A5 and SULT1A1 genes were amplified using upstream and downstream primers, Genotyping of CYP3A5 and SULT1A1 variant allele restriction fragment length polymorphisms was determine by using (Ssp1 and Hae 11) restriction enzymes

respectively. Separated products by a agarose gel electrophoresis wild type, heterozygous and mutant genotypes for CYP3A5 and SULT1A1 were visualized on UV transelimellimantor.

Results

Distribution of leukemia in patients shows choric myeloid leukemia (CML) 25 (25%), were chronic lymphocytic leukemia (CLL) 26(26%), acute myeloblastic leukemia (AML) (21) 21% and acute lymphoblastic leukemia (ALL) 28(28%) (Fig 1).

The CYP3A5 genotypes show wild type allele CYP3A5*1/*1, heterozygous allele CYP3A5*1/*3 and mutant allele CYP3A5*3/*3 patients and controls (Table 1).

The SULT1A1 genotypes shows wild type allele SULT *1/*1, heterozygous allele SULT SULT *1/*2 and mutant allele SULT *2/*2 in patients and controls (Table 2).

Related to type of leukemia the mutant CYP3A5*3/*3 frequencies in CML were 11(42.3%), CLL 8(30.8%) AML 3 (11.5%) and ALL 4 (15.4%). While the mutant SULT *2/*2 frequencies were 5(45.46%) in CML and 2(18.18%) in CLL, AML and ALL respectively (Table 3& 4).

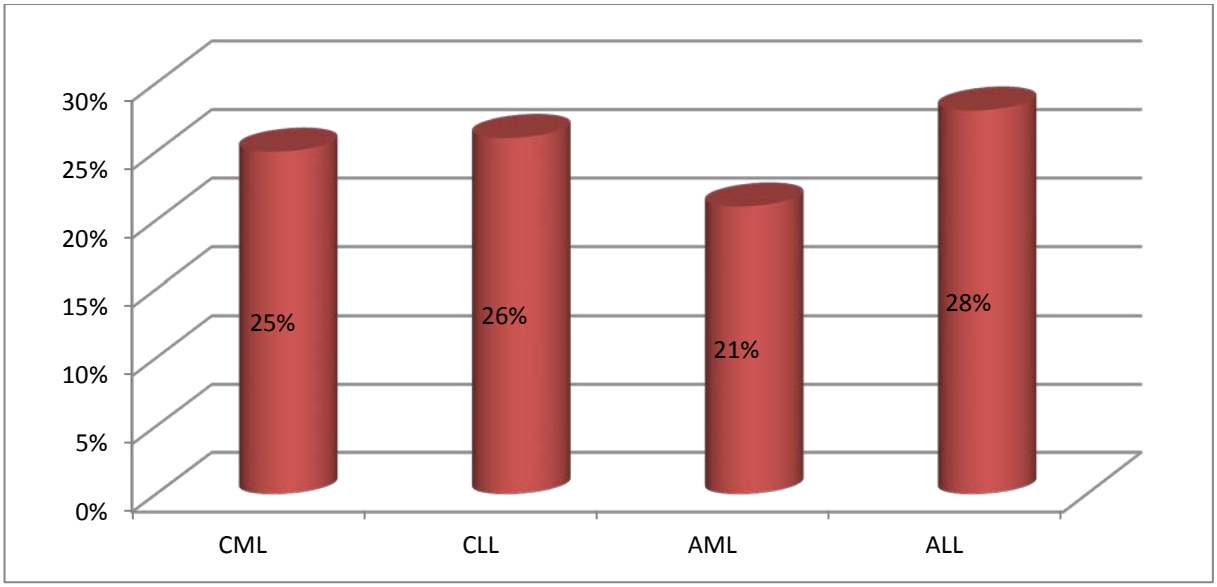

Figure 1. Distribution of leukemia in patients shows CML 25 (25%), CLL26(26%), AML (21) 21% and ALL28(28%).

Table 1. Frequency of CYP3A5 Genotypes in patients and controls

	CYP3A5 genotypes				Total
	Status	CYP3A5*1/*1 (Wild type)	CYP3A5*1/*3 (Heterozygous)	CYP3A5*3/*3 (Homozygous)	
	Patients	52	22	26	100
	Controls	83	8	9	100
	Total	135	30	35	200

Chi-square =21.9,df=2, P. value =0.001(significant)

Table 2. Frequency of SULT1A1 Genotypes in Patients and controls

	SULT1A1 genotypes				Total
	Status	SULT *1/*1 (Wild type)	SULT *1/*2 (Heterozygous)	SULT *2/*2 (Homozygous)	
	Patients	85	4	11	100
	Controls	90	4	6	100
	Total	175	8	17	200

Chi-square =16.13,df=2 and P. value=0.446(insignificant)

Table 3. Frequency of CYP3A5 alleles in different types of leukemia

	Type of leukemia	CYP3A5*1/*1 (Wild type)	CYP3A5*1/*3 (Heterozygous)	CYP3A5*3/*3 (Homozygous)	Total
	CML	10/52 (19.2%)	4/22(18.18%)	11/26 (42.3%)	25
	CLL	14/52(26.9%)	4/22(18.18%)	8/26 (30.8%)	26
	AML	10/52(19.2%)	8/22(36.37%)	3/26 (11.5%)	21
	ALL	18/52(34.6%)	6/22(27.27%)	4/26(15.4%)	28
	Total	52	22	26	100

Table 4. Frequency of SULT1A1 alleles in different types of leukemia

	Type of leukemia	SULT1A1*1/*1 (Wild type)	SULT1A1*1/2 (Heterozygous)	SULT1A1*2/*2 (Homozygous)	Total
	CML	20/85(23.5%)	00%	5/11(45.46%)	25
	CLL	23/85(27.1%)	1/4(25%)	2/11(18.18%)	26
	AML	17/85(20%)	2/4(50%)	2/11(18.18%)	21
	ALL	25/85(29.4%)	1/4(25%)	2/11(18.18%)	28
	Total	85	4	11	100

Discussion

In the present study, the association of molecular variables with type of leukemia was evaluated and primarily different frequencies of leukemia were determined in patients, ALL 28% as the highest and AML 21% as the lowest chronic leukemias CLL 26% and CML 25%, and more occurrence of leukemic in male 62% than in female 38% patients, higher incidence of ALL in male sex in contrast to AML in female, while chronic leukemias revealed higher incidence of CML and CLL in male patients than female, the findings in agreement with other studies reported that there is much variability in incidence of leukemia in different populations.[14] CYP3A5 polymorphism in the present study determined three genotypes CYP3A5*1/*1 wild type, CYP3A5*1/*3 heterozygous and significant increases in CYP3A5*3/*3 mutant genotype in patients with leukemia. Related to type of leukemia higher frequency of mutant CYP3A5*3/*3 genotype was found in patients with chronic leukemias compared to those with acute leukemias.

CYP3A5*3 polymorphism explained significant elevation of mutant 3/3 genotype allele among leukemic patients causes loss expression of CYP3A5 leads to drug toxicity effect and subsequent DNA damage which might be responsible for disease incidence. Hence loss of CYP3A5 expression associated with mutant allele.

Similar frequencies of CYP3A5*3/*3 allele in both the leukemic group and controls was reported. [15,16] On the other hand Shen et al., 2008[17] reported that the expression of CYP3A5 in patients with acute leukemia was closely associated with the chemotherapeutic effect and prognosis. However other reported a significant association of CYP3A5*3 polymorphism with solid tumors in some populations like those of the Indian subcontinent, the frequencies of mutant CYP3A5*3 alleles were elevated significantly in the CML group compared to controls (χ^2=93.15, df=2, p=0.0001). [18]

Substantial raise of mutant CYP3A5*3 allele frequency in CML population was detected and indicates the loss of CYP3A5 expression linked with altered allele might be accountable for the buildup of endogenous steroids or xenobiotics in various tissue which might leads to cancer and leukemia. [19] The study identified that SULT 1A1 polymorphism include three genotypes SULT 1A1 *1/*1 wild type, SULT 1A1 *1/*2 heterozygous and mutant genotype SULT 1A1 *2/*. Higher frequency of mutant SULT 1A1 *2/*2 genotype was observed in patients with CML and lower frequency was observed in patients with CLL, AML and ALL, while Vineis et al. [20] 2007 mention *that* SULT1A1 polymorphisms (phase II metabolism) modify the risk in ALL patients. Several studies have investigated the association of SULT1A1 genes variants with cancer or leukemia, and the results have been controversial. [21]

In conclusion, CYP3A5 variants exhibit significant risk and associated with leukemia particularly mutant type CYP3A5*3/*3 allele. Gender is an important modulator of the risk and may explain certain aspects related to the male/female incidence of leukemia. CYP3A5, and SULT1A1 mutations are important and can be used as markers to predict a person′s risk for leukemia.

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