CYP 3A5*3 Gene Polymorphism Among Sudanese Patients With Chronic Myeloid Leukemia

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Abstract

Introduction: Interaction of environmental and genetic elements plays a vital role in the pathogenesis of CML and other types of cancer. CYP 3A5 is enzyme responsible of metabolizing of approximately 50 % of therapeutic drugs and xenobiotics. So polymorphism variation may genetically predispose individuals to CML.

Objective: The purpose of this study was to determine CYP3A5*3 Polymorphism among Sudanese patients with Chronic Myeloid Leukemia (CML).

Materials and Methods: This case control study was conducted among 50 patients with chronic myeloid leukemia among both genders at different ages and 42 apparently healthy control subjects. The CYP3A5*3 genotype was determined using (PCR-RFLP) method.

Results: Paerson`s chi-square test was used to assess the possible link between CYP3A5*3 genotype and CML. The percentage of CYP3A5*3*3 genotype in CML patients was significantly higher than in control (OR = 11.71, 95% CI: 4.30 – 31.83; p =0.000). The frequency of mutant genotype was higher among CML patients with advance phase compared with chronic phase, 100% versus 80% respectively.

Conclusion: CYP3A5*3/*3 genotype associated with CML development and progression.

Introduction

Significant association between increased risk of chronic myeloid leukemia and different gene polymorphism in CYP 3A5 family is known. The study of these genes polymorphisms may help in better management and control of the disease. Leukemia is a group of [cancers](#) that usually begin in the [bone marrow](#) and result in high numbers of abnormal [white blood cells](#) ⁽¹⁾. These white blood cells are not fully developed and are called [blasts](#) or leukemia cells⁽²⁾. Symptoms may include bleeding and [bruising](#) problems, [feeling tired](#), fever, and an increased risk of infections⁽²⁾. These symptoms occur due to a lack of normal [blood cells](#). Diagnosis is made by testing of [blood](#) or [bone marrow biopsy](#)⁽²⁾.

The exact cause of leukemia is unknown. Different kinds of leukemia are believed to have different causes. Both [inherited](#) and environmental (non-inherited) factors are believed to be involved⁽³⁾. Risk factors include [smoking](#), [ionizing radiation](#), some chemicals (such as [benzene](#)), prior chemotherapy, and [Down syndrome](#)⁽³⁾⁽⁴⁾ People who have a family history of leukemia are at higher risk⁽⁴⁾. The main four types of leukemia are: [acute myeloid leukemia](#) (AML), [acute lymphoblastic leukemia](#) (ALL), [chronic myeloid leukemia](#) (CML) and [chronic lymphocytic leukemia](#) (CLL) as well as a number of less common types⁽⁴⁾⁽⁵⁾.

Chronic myeloid leukemia (CML)

CML (cancer of the [white blood cells](#)), is characterized by the uncontrolled increased growth of predominantly [myeloid](#) blood cells in the [bone marrow](#) and ultimately accumulation of these cells in the blood. CML is disorders of a clonal bone marrow [stem cell](#) in which a proliferation of mature [granulocytes](#) ([neutrophils](#), [basophiles](#) and [eosinophils](#)) and their precursors is found. It is a type of [myeloproliferative disease](#) associated with a characteristic [chromosomal translocation](#) called the [Philadelphia chromosome](#)⁽⁶⁾.

Phases of CML

CML is often divided into three phases based on clinical characteristics and laboratory findings. Before intervention, CML typically begins in the chronic phase, and after several years may progresses to an accelerated phase and ultimately to a blast crisis, the later phase is the terminal phase of CML and clinically behaves like an [acute leukemia](#)⁽⁷⁾

Table 1 Phases of Chronic Myelogenous leukemia defined by the World Health Organization (WHO) ⁽⁸⁾

CML phase	WHO definition
Chronic stable phase	Peripheral blood blasts less than 10% in the blood and bone marrow
Accelerated phase	Blasts 10-19% of white blood cells in peripheral and/or nucleated cells in bone marrow, persistent thrombocytopenia (<100×10 ⁹ /L) which is unrelated to therapy or persistent thrombocytosis (>1000×10 ⁹ /L) unresponsive to therapy,>20% basophiles in the blood or bone marrow increasing white blood cells and spleen size unresponsive to therapy, evolution of Cytogenetic with new abnormalities in addition to the Philadelphia chromosome
Blast crisis	Peripheral blood blasts ≥20% of white peripheral blood cells or nucleated bone marrow cells; extramedullary blast proliferation; and large foci or clusters of blasts on bone marrow biopsy

Cytochrome P 450/ 3A5(CYP3A5)

CYP3A5is the major drug – metabolizing enzyme in the gastrointestinal and liver ⁽⁹⁾. It has broad substrate specificity and can metabolize approximately 50% of therapeutic drugs (nifedipine and cyclosporine), steroid hormones (testosterone, progesterone and anderostenedione) and several xenobiotics⁽¹⁰⁾. It is highly inducible and can also be inhibited by numerous drugs. The CYP3A5 gene is a part of of cytochrome P450 genes: CYP3A4, 3A5, 3A43 and 3A7, located on chromosome 7q21.1 position ⁽¹¹⁾. CYP3A5 gene exhibit interindividual variations in expression levels. The most frequent polymorphisms identified in all ethnic populations involving splicing defects are CYP3A5*3 and CYP3A5*6 ⁽¹²⁾

Polymorphism of CYP3A5*3 in intron 3 of the CYP3A5 gene can reduce expression of CYP3A5 and to few than 1/1000 of that found in carriers of the wild type allele (CYP3A5*1) CYP3A5*3 allele (substitution of

A to G at position 6986) produces a cryptic splice site and encodes for an abnormal spliced mRNA with a normal mRNA, resulting in a high expression of this enzyme.

Since CYP3A5 is the primary extra hepatic CYP3A enzyme, the polymorphic variation in CYP3A5 might be influencing the depositing of endogenous steroids or xenobiotics in these tissues (kidney, prostate, lung, breast, and leukocytes) which might increase the risk to develop disease condition. ⁽¹³⁾

Materials And Methods

A cross sectional case control study conducted in 50 Sudanese patients with CML (25male and 25females) and compared with 42 apparently healthy (25male and 17 females) Sudanese individual as control. The study was conducted in Radiation and Isotope center in Khartoum (RICK) (from January to August /2017).

Sample collection

Blood Samples were collected from individuals upon their agreement. A total of 2.5 ml EDTA blood samples were obtained from CML patients and apparently healthy volunteers as Control.

DNA Extraction

DNA was extracted by Chelix (100) method protocol ^(14,15); Samples were stored at -20C until analysis, Gene quant device (Amersham bioscience – Biochrome LTD- Cambridge CB4 of J. England) used to detect the quantity of (DNA& Protein) and quality (Purity &ratio) of DNA.

Genotyping of CYP3A5*3polymorphism

CYP 3A5 polymorphism was determined with a polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP].

The PCR primers were: Sense primer: 5-CATCAGTTAGTAGACAGATGA-3, Antisense primer: 5-GGTCCAAACAGGGAAGAAATA-3 .

PCR was carried out in a total volume of 25 µl. It consist of 5µl of genomic DNA, 1µl from each primer, ready to load master mix (Maxime PCR premix series) and 13µl distilled water. PCR was initiated , initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 61°C for 1 minute, 72°C for 1 minute and a last extension at 72°C for 7 minutes(the same program for GSTT1 and GSTM1).

PCR products were analyzed on a 2% Agarose gel stained with 0.5 µg/mL ethidium bromide, and visualized by gel documentation system (to check the presence of 293 Pb of CYP 3A5)

Then the PCR product was digested with the restriction endonuclease Alw26I restriction enzyme {Thermo Scientific Alw26I # ER0031 (onebio)} as follow:

For each 10 µl of PCR product a 7.5 µl nuclease free water, 2 µl from 10X Tango buffer (#B19) and 0.5 µl from Alw26I restriction enzyme were added, then incubated at 37°C for 16 hrs, followed by incubation at 65°C for 20 minute to inhibit the enzyme activity. The products are then resolved on 2% agarose gel electrophoresis containing ethidium bromide, then visualized using UV transilluminator.

The amplified fragment after digestion with SSP1 restriction enzyme, will give rise to: 3 fragments at 20 bp and 125 and 148 bp indicating the presence of wild type (CYP 3A5*1/*1), appearance of 2 fragments at 168 bp and 125 bp indicates the presence of homozygous mutant type (CYP 3A5*3/*3), while presence of 4 fragments at 168 bp, 148 bp and 125 bp and 20 indicates the presence of heterozygous mutant type (CYP 3A5*1/*3). For quality control, genotyping of 10 (10.86 %) of the samples were repeated blindly and were identical to the initial results.

Statistical Analysis

Statistical analysis included description statistic of mean, standard deviation. Odds ratio (OR) with a confidence interval (CI) of 95% was calculated. The person's chi-square test was used to compare the genotype distribution between patients and control. Also independent t test have been used. P-value less than 0.05 were considered as statistically significant. We have used the statistical package SPSS version 21.

Results

A total of 50 patients diagnosed with chronic myeloid leukemia attended to the Radiation and Isotope Center of Khartoum (RICK) Sudan, their ages ranged between (16- 79) years, 25(50%) of them were males and 25(50%) were females, compared to 42 volunteers as control group their ages ranged between 18-50 years, 25(60%) of them were males and 17(40%) of them were females.

The statistical analysis showed that there was a significant difference in the frequency of (CYP 3A5*3/*3) gene between patients and control group, 42(76.4%) versus 13(23.6%) respectively. (OR = 11.71, 95% CI: 4.3 –31.83; p =0.000). While a significantly negative association was observed in the frequency of (CYP 3A5*1/*3) gene when compared between patients and control group, 8(22.86%) versus 27(77.14%) respectively. (OR = 0.08, 95% CI: 0.03 –0.23; p =0.000).

In contrast there was higher frequency of (CYP 3A5*3/*3) polymorphism among CML patients in advanced phase (8/8) (100%) compared to chronic phase (34/42) (80.95%).

Considering laboratory data, there was insignificance difference of mean of Hb, Platelet's count and WBCs count, when were compared between patients with homozygous mutation (CYP 3A5*3/*3) and other group of patient with heterozygous mutation (CYP 3A5*1/*1). Table 3

Regarding distribution of homozygous allele (CYP 3A5*3/*3) among Sudanese population, the higher frequency was observed among western population 15/28(53.57%), then central region 9/28 (32.14%)

while the least frequency of allele was determined among northern and eastern population 2/28(7.14%) for both group.

Table 2: Frequency of CYP 3A5 among case and control

Population			CYP 3A5			Total
			Homozygous (CYP 3A5*3/*3)	Heterozygous (CYP 3A5*1/*3)	Normal (CYP3A5*1/*1)	
Study groups	Case	Count	42	8	0	50
		%	84%	16%	0 %	100%
	Control	Count	13	27	2	42
		%	31%	64%	4.8%	100%

Table 3: Descriptive statistics of CYP 3A5 polymorphism and hematological parameters among CML patients

Parameter	Mean among Gene variant		p. value
	CYP 3A5*1/3*	CYP 3A5*3*3	
Hb (%)	76.00	75.87	0.99
Platelets (X10 ⁹ /L)	270000	294625	0.92
TWBCs (X10 ⁹ /L)	5200	5962	0.77

Independent t test was used to calculate P value

P value less than 0.05 considered significant

Table 4: CYP3A5 * Gender association

CYP3A5		Gender		Total
		Female	Male	
Normal	Count	2 _a	0 _a	2
	% within CYP3A5	100.0%	0.0%	100.0%
	% within Gender	4.8%	0.0%	2.2%
	% of Total	2.2%	0.0%	2.2%
Heterozygous	Count	14 _a	21 _a	35
	% within CYP3A5	40.0%	60.0%	100.0%
	% within Gender	33.3%	42.9%	38.5%
	% of Total	15.4%	23.1%	38.5%
Homo	Count	26 _a	28 _a	54
	% within CYP3A5	48.1%	51.9%	100.0%
	% within Gender	61.9%	57.1%	59.3%
	% of Total	28.6%	30.8%	59.3%
Each subscript letter denotes a subset of Gender categories whose column proportions do not differ significantly from each other at the .05 level.				

Discussion

Studies of genetic susceptibility of CML may help to identify populations at risk and clarify the mechanisms of disease. Genetic variants within genes that encode enzymes involved with metabolism such as CYP3A5 have been shown to increase the likelihood of developing various forms of cancers.

The present study found a significant association between (CYP3A5*3 and CML risk) OR = 11.7, 95% CI: 3.3 – 31.8 ; p = 0.00). Also 100% of patients with advance disease have the homozygous mutation compared to 80% in chronic phase, this finding was agreed with Indian study by K Sailaja et al (2010) suggested that CYP3A5*3 is associated with CML development and disease progression) $\chi^2 = 93.15$, df = 2, p = 0.0001 (.CYP3A5*3 (increase in patients with advance phase (71%) compared with chronic phase (65%).⁽¹⁶⁾ but this study was disagreed with study done by Blanco JG et al found no differences between

the control and t-ML group in the incidence of homozygous CYP3A5*3 genotypes: 82.0% vs. 85.4% in whites (P = 0.403), 6.5% vs. 12.5% in blacks (P = 0.508), and 69.6% vs. 75.0% in Hispanics (P = 0.663).⁽¹⁷⁾ also the study disagreed to Bajpai P1, et al from India (2010), demonstrated that no association was observed between CML and CYP3A5*1/1*, CYP3A5*3*3 or CYP3A5*1/3* (P value = 0.88, 0.65 and 0.80) CYP3 genotypes may not significantly modify the risk of CML development. ⁽¹⁸⁾

Disagreement with this study may relate to different factors like ethnic group, predisposing factors, geographical distribution and genetics

In this study the mutant genotype have no effect on gender distribution, this result was agreed to K Sailajaletal (2010) ⁽¹⁶⁾

Conclusion

CYP3A5*3/3* associated with CML development and progression while CYP3A5*1/3* appear to have protective factors against the disease, so early prediction of the polymorphism for all population can help in disease prevention or prediction.

Abbreviations

CYP: Cytochrom P; **DNA**: Deoxyribo Nucleic Acid; **EDTA**: Ethylene Diamine Tetra-Acetate; **CML**: Chronic Myeloid Leukeamia; **CLL**: Chronic Lymphocytic Leukeamia; **ALL**: Acute Lyphoblastic Leukeamai ; **AML**: Acute Myeloid Leukeamia; **SD**: Standard Deviation; **TWBC**: Total White Blood Cells; **Hb**: Haemoglobin.

Declarations

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Authors'contributions

All authors was contributing in writing and revising of paper; Elharam, Nazic and Wala Eldin were the main experimenter for the work, Salah was the co-worker for the experiment, **NassrEldin** was the main supervisor and reviewer of this research work.

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Availability of data and materials

Data will be available for public without any restriction, while materials will be available under request from corresponding author.

Ethics approval and consent to participate

This study had been approved by the faculty of medical laboratory sciences, Al-zaiem Al-azhary University. The purpose of this study had been clarified and discussed with the patients and their relatives. Samples had been taken after informed consent from hospital and patients.

Consent for publication

All authors consent for publication.

Competing interest

Not applicable

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