Sudan University of Sciences and Technology

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Detection of Polymorphism in Cytochrome P450 (*CYP3A5*) and Sulfertranseferase *(SULT1A1*) Genes in Patients with Leukemia in Sudan

الكشف عن الصور الجينية المتعددة للسايتوكروم (P450) جين (*CYP3A5*) والسلفوترانسفيريز جين (*SULT1A1*) للمصابين بسرطان الدم في السودان

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قال الله تعالى :

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ

سورة التوبة الآية105

Dedication

I dedicate this work to my extended family

Also I dedicate this work to every person who takes me valuable things

Acknowledgment

First praise to Allah who help me the strength and patience to accomplish this work. My thanks and extracted due to my supervisor Dr. Mahmoud Mohamed Elgari for his kind direction, guidance, rare and care to complete this work.

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Abstract

This is a case - control study done to investigate genetic differences among Sudanese patients with leukemia and 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), and 100 healthy individuals as control group. patients were fully informed about the aims of the study, besides approved written consent. DNA extracted from EDTA blood samples and PCR-RFLP performed for each patient and control subject. two primer pairs were designed according to published sequence data for, intron 3 in CYP3A5, exon 7 in SULT1A1.The different genotypes were scored for each locus and data were analyzed using computer software SPSS (versoin17). Distribution of leukemia among patients revealed that 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%). 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.

In conclusion, CYP3A5 variants exhibit significant risk 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

male / female incidence of leukemia.

المستخلص

هذه الدراسة حالة ضبط لتحديد الاختلافات الجينية بالنسبة لمرضي سرطان الدم بالسودان مقارنة بمتطوعين اصحاء كمجموعة قياسية, تم استخدام الجينات عديدة الاشكال كمؤشرات وراثية واستعمال طريقة التحليل الجيني المعتمدة على التفاعل البولميري المتسلسل في تحديد النمط الوراثي. كما هدفت الدراسة الى معرفة دور التباين الجيني في خطر الاصابة بسرطان الدم في السودان لذلك تمت دراسة الجينات التي تعمل على هدم السموم في الانسان, لهذا الغرض تم اختيار الجين (CYP3A5) والجين (SULT1A1). شملت الدراسة (١٠٠) مريض بسرطان الدم و(١٠٠) متطوع أصحاء ظاهريا كمجموعة ضابطة وذلك في الفترة من (١٢.٢-١٥.٢). تم جمع عينات الدم من جميع الافراد ومختلف الاعمار بعد التوقيع علي اقرار مكتوب فيه تعريف بالدراسة واهميتها . ثم استخلص الحامض النووي ناقص الاكسجين الDNA وتم تحليله في مواقع الجينات عديدة الاشكال باستعمال طريقة التحليل المعتمدة في تحديد النمط الوراثي . كما تم رصد وتحليل البيانات الناتجة احصائيا. لم تظهر هذه الدراسة اي اختلاف بين المرضى والاصحاء في التوزيع التكراري للاشكال الشاذة لجينات ((SULT1A1) بينما اظهرت الدراسة اختلافات بنسبة كبيرة بين المرضى والاصحاء في التوزيع التكراري للاشكال الشاذة للجينات التي تعمل على ايض السموم (CYP3A5), اظهرت الدراسة ان الذكور اكثر عرضة للإصابة بمرض سرطان الدم مقارنة بالإناث. نسبة الإصابة بالمرض في الذكور ٦٢ ٪ وتوزيعها التكراري كالآتي سرطان الدم المايلودي المزمن نسبة الإصابة بالنسبة للذكور ٦٤ ٪ وسرطان الدم اللميفاوي المزمن ٥٤ ٪ وسرطان الدم المايلودي الحاد ٤٨ ٪ و سرطان الدم اللميفاوي الحاد ٧٩ ٪ سرطان الدم اللميفاوي المزمن, وكانت نسبة الإصابة بمرض سرطان الدم بالنسبة بالاناث ٣٨٪ وتوزيعها التكراري كالآتي سرطان الدم المايلودي المزمن ٣٦ ٪ وسرطان الدم اللميفاوي المزمن ٤٦ ٪ وسرطان الدم المايلودي الحاد ٥٢ ٪ وسرطان الدم اللميفاوي الحاد ٢١ ٪. كما أظهرت الدراسة أن المجموعات العرقية الافرواسيوية اكثر تكرار مقارنة بالمجموعات العرقية الأخرى.

List of abbreviations

AA	Afro Asiatic
AEL	Acute Elytroid Leukemia
BCR /ABL	Breakpoint-Cluster Region – Abelson
CML	Chronic Myeloid Leukemia
DNA	Deoxy Nucleic Acid
CYP3A5	Cytochrome 3A5 Enzyme
CYP450	Cytochrome P450
EBV	Epstein-Barr Virus
EDTA	Ethylene Di-amine Tetra Acetic Acid
FAB	French-American-British Classification
GST	Glutathione S-transferase
HTLV-1	HumanT-cell Lymphoma/ Leukemia Virus
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAT	N-Acetyl Transferase
NS	Nilo Saharan
NK	Niger Kango
Ph	Philadelphia Chromosome
RFLP	Restriction Fragment Length Polymorphism
RT	Room Temperature
RICK	Radiation and Isotopes Centre Khartoum
SNPs	Single Nucleotide Polymorphisms
SULT	Sulphotransferase
SUST	Sudan University of Sciences and Technology
TBE	Tris Buffer EDTA
UGT	Uridine diphospho Glucuronosyl Transferase
PCR	Polymerase Chain Reaction

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Chapter One

Introduction and literature review

Chapter One

1. Introduction and Literature Review

1.1. Introduction

Malignancy is a term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is a cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord. Also called malignancy (ACS, 2013). Xenobiotic biotransformation is a key mechanism for maintaining homeostasis during exposure to various xenobiotic, such as drugs, industrial chemicals or food (Petra *et al.,* 2010). In the human genome there are at least 3.1 million single nucleotide polymorphisms (SNPs), or about 1 SNP per kilo base of sequence (Frazer *et al*., 2007*).* Functional polymorphisms in the genes encoding xenobiotic-metabolizing enzymes cause inter individual differences that contribute to leukemia susceptibility. Cytochrome P450, which belongs to phase I 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 (Zanger, 2004).

1.2.1. Leukemia

European physicians in the 19^{th} century were the earliest observers of patients who had markedly increased white cell counts. The term Weisses

Blut “white blood” emerged as a designation for disorder **(**Ball *et al.*, 2003**).**

Leukemia (American English) or leukaemia (British English) comes from the Greek leukos - white, and haima – blood that discovered by Dr. Alfred Velpeau in France in1827, Named after by pathologist Rudolf Virchow in Germany, 1845. Leukemia is a group of malignant disorders affecting the blood and blood-forming tissues in the bone marrow, lymphatic system, and spleen. The word leukemia literally means “white blood” because it is a neoplastic proliferation of one type of blood cell, typically a leukocyte or white blood cell. Leukocytosis is an increased of white blood cell count as normal response to infection, but when leukocytosis becomes chronic or progressively elevates without obvious cause, then it may indicate malignancy **(**Lockwood *et al.*,2013). Leukemia is defined as a disease where a clone of a certain cell (malignant) gains an uncontrolled growth (proliferation) advantage over the normal cells, leading to an overwhelming abundance of that malignant cell. In the case of leukemia, this uncontrolled proliferation leads to the predominance of the malignant cell in the bone marrow followed by its spread to peripheral blood, and possibly other tissues (Mirza, 2008)**.**

1.2.2. Incidence of Leukemia

There were an estimated 14.1 million cancer cases around the world in 2012, out of these, 7.4 million cases were in men and 6.7 million in women. (Ferlay J *et al.*, 2012). Lung cancer was the most common cancer worldwide contributing 13% of the total number of new cases diagnosed in 2012. Breast cancer (women only) was the second most common cancer with nearly 1.7 million new cases in 2012.Colorectal cancer was the third most common cancer with nearly 1.4 million new cases in 2012

(Ferlay J, 2012). Breast cancer (women only) was the most common cancer In Sudan contributing 34% of the total number of new cases diagnosed in 2014. Prostate cancer was the second most common cancer with nearly 11% of new cases in 2014. Esophagus cancer was the third most common cancer with 9% of new cases in 2014, and ovary is the fourth common one in Sudan **(**RICK, 2014*).*

1.1.3. Frequency of all type of Leukemia in Sudan

About 165 Sudanese diagnosed with AML in 2014.male 95 female 70 most (about 45) 65 or older, and about 16 children and tens will get this disease. More than 164, Sudanese will be diagnosed with ALL in 2014.male 104 female 60 Most (more than, 50) children and teens. About 233 Sudanese diagnosed with CML in 2014. Male 139 female 94, almost half (about 50) are 65 or older, and only about 9 children and teens will get this disease. About 121 Sudanese diagnosed with CLL in 2014.male 88 female 33 most (about 100) 65 or older. This disease almost never affects children or teens **(**RICK, 2014*).*

1.1.4. Risk Factor of Leukemia

The origin of leukemia is not clear, but it may result to a combination of factors, which can include genetic predisposition, chromosomal changes, chemical agent's benzene, chemotherapeutic agents, radiation, and immune compromise (lockwood *et al*., 2013).The risk of the disease increases in individuals with Down syndrome, Fanconi's syndrome, ataxia-telangiectasia, Bloom's syndrome, or some other forms of congenital aneuploidy and in an identical twin of a leukemia victim (Mosby's, 2009). Two viral diseases have been implicated in the development of leukemia. One is the Epstein-Barr virus (EBV) the other is the human T-cell lymphoma/leukemia virus (HTLV-1) (Lockwood *et al*., 2013).

1.1.5. Classification of Leukemia

Leukemia is classified according to the predominant proliferating cells. There are several different types of leukemia. The types of leukemia are first divided according to the type of stem cell. Myelogenous leukemia develops from abnormal myeloid cells. Lymphocytic leukemia develops from abnormal lymphoid cells. The types of leukemia are further grouped according to how quickly the leukemia develops and grows to acute or chronic (Lockwood *et al.,* 2013). Leukemia can be classified into four main types include Acute lymphocytic leukemia (ALL), it is the most widespread category in young children and so it gets adults. It is affected 3,800 new cases every year. Acute myeloid leukemia (AML) affects children and adults, its incidence about 10.600 new cases of leukemia each year. While chronic leukemia usually occurs in adults. Which involve Chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) affect about 4.400 -7.000 novel cases of leukemia every year respectively. Chronic myeloid leukemia has the ability to extension in myeloid and lymphoid lineage (Lockwood *et al.,* 2013). Acute leukemia starts suddenly developing within days or weeks. In acute leukemia, the bone marrow cells cannot mature properly immature leukemia cells continue to reproduce and build up. Without treatment, most people with acute leukemia would live only a few months. Some types of acute leukemia have a less favorable outlook respond well to treatment, and many patients can be cured (Byrd *et al*., 2014).Chronic leukemia develops slowly over months or years, and may not cause any symptoms early in the disease. Symptoms start to appear as the number of leukemia cells in the blood or bone marrow increases in chronic leukemia, the cells can mature partly but not completely. These cells may look fairly normal, but they generally do not fight infection as well as

normal white blood cells do. They also live longer, build up, and crowd out normal cells. Chronic leukemia tend to progress over a longer period of time and most people can live for many years. But chronic leukemiais generally harder to cure than acute leukemia **(**Mosby's, 2009).

1.1.6. Acute lymphocytic leukemia (ALL)

ALL is stem disorder in which the bone marrow produces an increased number of blasts lymphoblast that accumulates and eventually spill into circulation. It is thought that the disease begins with genetic mutations that occur in hematopoietic stem cell. These mutations accumulate in the stem cell to produce three general functional impairment increased rate of proliferation by increasing self- renewal and resistance to negative growth controls, loss of differentiation beyond the blast stageandreduced apoptotic death. Acute lymphocytic leukemia (ALL) is the most common cancer in childhood, although it can also occur in adults **(**Mohamed *et al.*, 2006**)**.

Although acute lymphoblastic leukaemia can be readily sub classified according to the many steps of normal B-cell and T-cell differentiation the only findings with therapeutic importance are T-cell mature B-cell, and B-cell precursor phenotypes **(**Tom, 2010**).** There are several systems of classification of acute lymphoblastic leukemia. The classification is commonly used to determine treatment and predict the prognosis of the cancer French-American-British (FAB) classification The French-American-British (FAB) classification that was used commonly earlier includes L1 – Around 25 to 30% of adult cases and 85% of childhood cases of ALL are of this subtype. In this type small cells are seen with regular nuclear shape homogeneous chromatin small or absent nucleolus scanty cytoplasm L2 – Around 70% of adult cases and 14% of childhood cases are of this type. The cells are large and or

varied shapes with irregular nuclear shape heterogeneous chromatin large nucleolus L3 – This is a rarer subtype with only 1 to 2% cases. In this type the cells are large and uniform with vacuoles (bubble like features) in the cytoplasm overlying the nucleus. This classification was abandoned by the World Health Organization because the L1 and L2 subtypes could not be differentiated in terms of clinical symptoms, prognosis and genetic abnormalities. The mature B-cell ALL or L3 type is now classified as Burkitt's lymphoma/leukemia as revised version of FAB classification. WHO proposed a classification of ALL that was to be the revised version of the FAB classification? This used the immune phenotypic classification that includes Acute lymphoblastic leukemia/lymphoma or formerly L1 and L2 (Henry, 2010).

1.1.7. Acute myeloid leukemia (AML)

Acute myeloid leukemia (AML) goes by many names, including acute myelocytic leukemia, acute myelogenous leukemia, acute granulocytic leukemia, and acute non-lymphocytic leukemia (Cancer Facts & Figures 2013). Acute myeloid leukemia (AML) is a heterogeneous group of leukemia's that arise in precursors of myeloid, erythroid, megakaryocytic, and monocytic cell lineages. This leukemia's results from clonal transformation of hematopoietic precursors through the Acquisition of chromosomal rearrangements and multiple gene mutations (Rubnitz *et al.,* 2007). Acute erythroid leukemia (AEL) is a rare form of acute myeloid leukemia (AML). It accounts for less than 5% of all AML cases **(**Arber *et al.,* 2008). The acute myeloid leukemia account for 20% of patients with leukemia in Europe, the USA and other western countries. The majority of affected individuals are adults and acute myeloid leukemia accounts for 85% of acute leukemia in adults. Acute myeloid leukemia's are only responsible for 20% of leukemia's in childhood.

Most patients with acute myeloid leukemia have cytogenetic abnormalities involving different chromosomes including deletion of long arm of chromosome 5, deletion of long arm of chromosome 7, trisomy 8, t (8;21) t(15;17) (Gordon K, 1990).

1.1.8. Chronic lymphocytic leukemia (CLL)

Chronic lymphocytic leukemia (CLL) a neoplastic disease characterized by the accumulation of small, mature appearing lymphocytes in the blood, marrow and lymphoid tissues. The first descriptions of patients with CLL were published in the early nineteenth century (Henry, 2010). In the 1840s Virchow described two forms of chronic leukemia that probably correspond to Chronic lymphocytic leukemia and chronic myelogenous leukemia **(**Klaassen *et al.,* 2010). Patients with the former were noted to have mild-to-moderate splenic enlargement, lymphadenopathy, and large numbers of small granular cells in the blood that resembled those found in enlarged lymph nodes. Virchow considered this type of leukemia to be principally related to disease of the lymph nodes rather than of the spleen. In 1893, Kundrat introduced the term lymphosarcoma to describe an indolent disease that affected lymph nodes. (Thomas, 2009). Histochemical staining techniques introduced by Ehrlich at the turn of the twentieth century made it possible for pathologists to distinguish between myeloid and lymphocytic leukemia. These methods enabled Turk in 1903 to establish a relationship of the leukemic cells in CLL to those in lymphosarcoma. He proposed the term lymphomatoses to describe several disorders, including CLL. Owing to its indolent nature, CLL was considered a “benign” lymphomatosisin 1924, Minot and Isaacs described the natural history of 98 patients with CLL, 26 challenging the notion that CLL was a “benign” process. In 1967, Dame shek hypothesized that CLL was an accumulative disease of

immunologically incompetent lymphocytes in the early 1970s, the leukemic cells from most cases of CLL were found to express surface immunoglobulin **(**Thomas, 2009). Doctors have found that there seem to be two different kinds of CLL. One kind of CLL grows very slowly and so it may take a long time before the patient needs treatment. The other kind of CLL grows faster and is a more serious disease. The tests look for proteins called ZAP-70 and CD38. If the CLL cells contain low amounts of these proteins, the leukemia tends to grow more slowly (American Cancer Society, 2013). According to the 2008 International Workshop on CLL (IWCLL) guidelines, the diagnosis of CLL requires a circulating B-lymphocyte count greater than or equal to 5 x 10^{9} /L in the peripheral blood, for the duration of at least 3 months **(**Mossatfa *et al.*, 2005).

1.1.9. Chronic myeloid leukemia (CML)

Chronic myeloid leukemia (CML) is a hematopoietic malignancy characterized by the presence of the Philadelphia (Ph) chromosome, In 1960, Peter C. Nowell, of the University of Pennsylvania School of Medicine, and David Hungerford, of the Fox Chase Cancer Center's Institute for Cancer Research described a translocation of chromosomes 9 and 22 (the Philadelphia chromosome, or (Ph) (j. Pediatr, 2008). specific chromosomal, Abnormality that is the result of reciprocal chromosomal translocation [t(9;22)(q34;q11)]. The translocation leads to rearrangement between the breakpoint-cluster region (BCR) and Abelson (Abl) genes. (BCR-ABL) is believed to play central role in the initial development of CML. The BCR-ABL gene encodes protein (p210) BC-RABL with deregulated tyrosine kinase activity. This protein contains NH_2-terminal domains of BCR and the COOH-terminal domains of ABL. Another fusion protein, p190, may be produced, but this is fusion protein, p190, may be produced, but this is leukemia (ALL).The oncogenic potential of

the BCR-ABL fusion proteins has been validated by their ability to transform hematopoietic progenitor. The BCR-ABL fusion gene encodes Bcr-Abl, an oncogenic fusion protein, a constitutively active tyrosine kinase, because the auto inhibition of the Abl kinase is disrupted by protein fusion **(**Huang, 2011). Bcr-Abl is a potent onco protein that facilitates leukemo genesis through several signal transduction pathways involving phosphatidylinositol-3 kinase (PI3K) and Akt, Janus kinase (Jak2) and signal transducer and activator of transcription (STAT), and Ras. Activation of these pathways in CML progenitor cells leads to increased proliferation and differentiation and to decreased apoptosis. Chronic myelogenous leukemia (CML) accounts for 15% of adult leukemia's. The median age of disease onset is 67 years; however, SEER statistics show that CML occurs in all age groups. An estimated 4870 cases were diagnosed in the United States, and 440 patients died of the disease. About 6,000 Americans will be diagnosed with CML in 2013(Huang *et al.,* 2011*).* CML occurs in 3 different phases (chronic, accelerated and blast) and is usually diagnosed in the chronic phase. However, gene expression profiling the accelerated and blast phase. Most of the genetic changes in progression occur during the transition from chronic to accelerated phase (J.Pediatr, 2008).

1.2. Literature Review

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 (Hatagima, 2002). Functional polymorphisms in the genes encoding xenobiotic-metabolizing enzymes cause inter individual differences that contribute to leukemia susceptibility. Cytochrome P450, which belongs to phase I biotransformation enzymes, is responsible for the metabolism of endogenous as well as exogenous DNA-reactive chemical compounds and xenobiotics which might induce gene toxicity and increase the risk for leukemia (Zanger, 2004**)**. 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(Paulussen,2000).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 (Hoskins, 2009). There are four genes in the CYP3A family (Goetz, 2011). In contrast a huge number of investigations were carried out to find the consequence of genetic variation of CYP3A4 and CYP3A5both are most commonly involved in drug related reactions (Regan, 2012). 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 (Kuehl, 2001). Meta-analyses indicate that the deletion of GSTM1 and GSTT1 is associated with a slightly increased risk of lung cancer and acute leukemia. 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. Transferase enzymes such as Sulfotransferase 1A1are 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 (Dooley *et al*,. 2000). 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 (Nagar, 2006). 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 (Nagar, 2006). There are many isoforms of the SULTs supergene family, each with different amino acid sequence identity and substrate specificity (Glatt, 2000). SULT1A1 is an important member of the sulfotransferase family involving in the pathogenic process of various cancers and leukemia (Au, 2009).

1.2.1. Detoxification

Detoxification specific metabolic pathway, which will be referred to as metabolic detoxification involves a series of enzymatic reactions that neutralize and solubilize toxins, and transport them to secretary organs (like the liver or kidneys), depends on multiple nutrients, cellular energy and a well-functioning body.

1.2.1.1. Xenobiotics metabolism

Detoxification of xenobiotics is critical in order to protect against toxic stress. Human detoxification is a complex, multi-reactive process that occurs primarily in cells of the liver and gastrointestinal wall and depends on multiple nutrients, cellular energy and a well-functioning body. Simply stated, most xenobiotics are taken from the blood stream through a 2-phase transformation that makes them progressively less toxic and more water-soluble for excretion from the body for successful detoxification **(**Journal of Oncology, 2011).

1.2.1.2. Phase I metabolism - activation

Phase I metabolism results in small chemical changes that make a compound more hydrophilic, 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 **(**Jennifer, 2012).

1.2.1.3. Phase II metabolism- conjugation

This is called the conjugation pathway, whereby the liver cells add another substance (eg.cysteine, glycine or a sulphur molecule) to a toxic chemical or drug, to render it less harmful. This makes the toxin or drug water-soluble, so it can then be excreted from the body via watery fluids such as bile or urine. Individual xenobiotics and metabolites usually

follow one or two distinct pathways. There are essentially six phase II detoxification pathways these include glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. In general, the respective conjugates are more hydrophilic than the parent compounds **(**Petra*et al.,* 2010). 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. Often, the functional groups generated in phase I reactions are required for attachment of the phase II polar groups (though in some cases phase II reactions can occur on their own). Transferase enzymes are responsible for most phase II reactions, e.g. uridine di phosphor glucuronosyl transferase (UGT), N-acetyl transferase (NAT), glutathione S-transferase (GST), and sulphotransferase (SlT) (Jennifer,2012).

1.2.2. Cytochrome P450

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. CYP450 enzymes are so named because they are bound to membranes within a cell (cyto) and contain a haeme pigment chrome and P that absorbs light at a wavelength of 450 nm **(**Tom,2010). The term 'cytochrome P450' was coined in 1962 as a temporary name for a colored substance in the cell. This pigment, when reduced and bound with carbon monoxide, produced an unusual absorption peak at a wavelength of 450 nm. Cytochrome is a misnomer given that the CYP450s are enzymes rather than true cytochromes. Despite this, the name 'cytochrome P450' has stuck and is so widely accepted that any change would be impractical **(**J Pharm, 2008).

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. Cytochrome P450 (CYP) enzymes are a superfamily of mono-oxygenises that are found in all kingdoms of lifeIn mammals, these enzymes are found primarily in the membranes of the endoplasmic reticulum (microsomes) within liver cells (hepatocytes), as well as many other cell types. These enzymes use haem iron to oxidise molecules, often making them more water-soluble for clearance they achieve this by either adding or unmasking a polar group (Jennifer, 2012).

1.2.2.1. Cytochrome P450 enzymes and gene

CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses However, the enzymes have not been found in E. coli. More than 21,000 distinct CYP proteins are known **(**Klaassen *et al*., 2010). There are more than 50 CYP450 enzymes, but the CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 enzymes metabolize 90 percent of drugs (Tom Lynch). There are 57 active CYP genes in the human genome, which are divided into 18 families. The first three families (CYP1– 3) are generally involved in the metabolism of exogenous substances such as drugs, whereas CYP families with higher numbers are usually involved in the metabolism of endogenous substances. CYP enzymes are responsible for 75– 80 per cent of all phase I-dependent metabolism and for 65–70 per cent of the clearance of clinically used drugs. Variation in CYP genes results in phenotypes classically defined as ultra-rapid, extensive, intermediate and poor metabolisers . An ultra-rapid metaboliser generally carries duplicated or multi-duplicated gene copies of the same allele,

whereas intermediate and poor metabolisers characteristically carry one and two defective alleles (eg gene inactivation or deletion), respectively. The term extensive metaboliser is normally used for subjects carrying two alleles giving normal activity of the CYP enzyme (also called the *1 or consensus allele). The metaboliser phenotypes are mainly used for describing drug metabolism, but genetic variation in CYPs with endogenous functions, such as in sterol, steroid, bile acid and fatty acid homeostasis, have also been well characterized, some of which give rise to disease states (Henery, 2010).

1.2.2.3. CYP (3A5) Gene

This gene, CYP3A5, Cytochrome P450, family 3, subfamily A, polypeptide 5 encodes a member of the cytochrome P450 superfamily of enzymes. This gene is part of a cluster of cytochrome P450 genes on chromosome 7q21.1. This cluster includes a pseudo gene, CYP3A5P1, which is very similar to CYP3A5. This similarity has caused some difficulty in determining whether cloned sequences represent the gene or the pseudo gen (CCR, 2005).Four CYP3A iso enzymes have been identified, CYP3A4, CYP3A5, CYP3A7 and CYP3A43, among which, CYP3A4 and CYP3A5 are the major components **(**Azarpira, 2006).CYP3A5 is expressed heterogeneously among the population and accounts for a great part of total CYP3A enzyme content in the intestine and liver. CYP3A5 is also expressed abundantly in the liver and small intestine, but only in the 30% of whites and 70% of blacks who possess CYP3A expression in the kidney is modest compared with the liver and is predominantly CYP3A5 **(**Haehner *et al*., 1996).

1.2.3.Sulfotransferase 1A1 (SULT1A1) enzymes and gene

Sulfotransferases (SULTs), a family of multi-functional enzymes, catalyze sulfonate conjugation. This is an important pathway in the

metabolism of several chemicals that are exogenous (e.g. mutagens from diet and environment) or endogenous (e.g. hormones and neurotrans-mitters). In addition to its important role in metabolic detoxification (Shen *et al.*, 2008). Sulfotransferaseisan enzyme that catalyze the transfer of a sulfate group from 3'-phosphoadenylyl sulfate (active sulfate) to the hydroxyl group of an acceptor, which produces the sulfated derivative and 3'-phosphoadenosine 5'-phosphate. The enzymes are also involved in both the posttranslational sulfation of proteins and sulfate conjugation of exogenous chemicals and bile acids (Medical dictionary, 2005). SULTs have a wide tissue distribution and act as a major detoxification enzyme system in adult and the developing human fetus. Two broad classes of SULTs have been identified membrane bound SULTs that are located in the Golgi apparatus of the cell and are responsible for the sulfonation of peptides (e.g., CCK), proteins, lipids and glycosaminoglycans affecting both their structural and functional characteristics(Falany, 1997; Negishi *et al*., 2001). Andcytosolic SULTs that are responsible for the metabolism of xenobiotics and small endogenous substrates such as steroids, bile acids and neurotransmitters (Blanchard *et al.*, 2004**;** Rikke and Roy, 1996; Nagata and Yamazoe, 2000). In humans three SULT families, SULT1, SULT2, and SULT4, have been identified, that contain at least thirteen distinct members. Nine crystal structures of human_cytosolic SULTs have now been determined. The universal sulfonate donor for these reactions is 3’-phosphoadenosine 5’-phosphosulfate (PAPS) and the transfer of sulfonate($SO3^-$) to a hydroxyl or amino- group is catalysedby a super gene family of enzymes called sulfotransferases (SULTs) (Niranjali *et al*., 2005).

1.2.4. Sulfotransferase Gene (SULT1A1)

Sulfotransferase 1A1 (SULT1A1) a major sulfotransferase enzyme in human beings. It is an important component in the detoxification pathway of numerous xenobiotics. The enzyme also plays an important role in the metabolism and bioactivation of many dietary and environmental mutagens, including heterocyclic amines implicated in carcinogenesis of colorectal and other cancers. The SULT1 family comprises of 9 members divided into 4 subfamilies (1A1, 1A2, 1A3, 1A4, 1B1, 1C1, 1C2, 1C3 and 1E1). SULT2A (SULT2A1) and SULT2B (SULT2B1a and SULT2B1b) belong to SULT2 family. The SULT4A1 and SULT6B1 are the only members of the SULT4 and SULT6 family, respectively (Lindsay*et al.,* 2008). SULT1A1 is one of the most important members in this enzyme family due to its extensive tissue distribution and abundance. This enzyme has a substantially higher activity than other SULTs in catalyzing the sulfonation of 4-nitrophenol, a commonly used assay in biochemical pharmacogenetic studies for testing the activity of thermo stable phenol SULTs (1–3). SULT1A1 catalyzes the sulfonation of estrogens to form water-soluble and biologically inactive estrogen sulfates, reducing the level of estrogen exposure in their target tissues (Weinshilboum, 1997).

1.2.5. Genetic Polymorphisms

A combination of the Greek words poly (meaning multiple) and morph (meaning form), this term is used in genetics to describe the multiple forms of a single gene that can exist in an individual or among a group of individuals. Polymorphism involves one of two or more variants of a particular DNA sequence. The most common type of polymorphism involves variation at a single base pair. Polymorphisms can also be much larger in size and involve long stretches of DNA. Called a single nucleotide polymorphism, or SNP (pronounced "snip"); scientists are

studying how SNPs in the human genome correlate with disease, drug response, and other phenotypes (Medical dictionary, 2006).

1.2.6. Genetic polymorphisms and risk of leukemia

Genetic polymorphisms in metabolic enzymes are associated with numerous cancers. There is a relationship between genetic polymorphisms of phase I metabolic enzymes including cytochrome P450 (CYP1A1), CYP2D6 and phase II metabolic enzymes such as glutathione S-transferees M1 (GSTM1) and GSTT1 and leukemia. Over the last decades, several studies have demonstrated that cancer cells have a unique metabolism compared to normal cells (Herling *et al.,* 2011). Metabolic changes occurring in cancer cells are considered to be fundamental for the transformation of normal cells into cancer cells and are also responsible for the resistance to different types of chemotherapeutic drugs (Khan, 2006).

1.2.7. Genetic polymorphisms in SULTS (1A1)

Genetic polymorphism was detected in many SULT forms such as the SULT1A1, SULT1A3, SULT1C2, SULT2A1, SULT2A3 and SULT2B1 enzyme. This mutation results in a variation of SULT1A1 thermal stability and enzymatic activity. Several authors have claimed that SULT1A1 polymorphism might play a role in the pathophysiology of lung cancer Urothelial carcinoma (Huang, 2009). And meningiomal brain tumors. Genetic polymorphisms in the human SULT1A1 gene define three alleles, SULT1A1*1, *2 and *2 (Henery *et al*., 2006).

1.3 Previous studies

CYP3A5 and SULT1A1 was an important enzyme in xenobiotic metabolism because it had broad substrate specificity with a high affinity for many compounds. The available data and studies have identified

correlates between expression of various metabolizing enzymes with risk of malignancies known to be induced by their substrates.MawadaEltayebstudyGlutathioneTransferases Mu1 and Theta1in Sudanese patients with Leukaemia (Tag, 2009). Other independent studies on various types of cancer have also shown this relationship between xenobiotic metabolizing enzymes and lung cancer (Lan *et al*., 2000), bladder cancer (Anwar *et al*., 1996; Kempkes *et al*., 1996).

1.4. Rationale

Some previous altitudes were done in this problem and this study may be addition to these studies. Also the result of this research may show the frequency of CYP3A5 and SULT1A1 genes polymorphisms in patients of different type of leukemia in Sudan.

1.5. Objectives

1.5.1. General Objectives

Detection of Polymorphism in Cytochrome P450 (CYP3A5 6986A>G) and Sulfertranseferase (SULT1A1 Arg213His) Genes in Leukemia Patients in Sudan.

1.2.2. Specific Objectives

1. To assess the most common type of leukemia in Sudan.
2. To study the genetic differences in carcinogen metabolizing genes in Sudanese leukemic patients compared to healthy controls.
3. To correlate between the mutant CYP3A5 and SULT1A1 genes and the risk of Sudanese leukemia Patients.

Chapter Two

Material and Methods

Chapter Two

Material and Methods

2.1. Study area

Hospital case control study was conducted in Radiation and Isotopes Centre, Khartoum, Sudan (RICK). Between the periods from 2012 to 2015. Molecular biology tests were conducted in National Central Laboratory Health, Khartoum, Sudan.

2.2. Study designing and sampling

The study samples were taken by choosing 200 persons ethically approved by Research Committee of the Sudan University of Science and Technology. A total of 100 patients with leukemia were enrolled and 100 healthy individuals or with family history of leukemia or any others cancers were included as control group.

2-3 Sample size

In this study sample size was calculated to achieve 100 blood samples from leukemia patients according to the sex, age, and tribe. The sample size was determined according to the following equation

$$S = \frac{n*z^2 * p(1-p)}{\{\{n*d^2\} + * p\,(1-p)\}}$$

S= population size

z=standard score

d=error rate

P=neutrality proportion

2.4. Inclusion Criteria

The criteria of choosing patients with different types of leukemia in this study was that, all patients diagnosed with routine tests and confirmed by histochemical methods and they were positive for leukemia, all age groups and both genders were included in this study.

2.5. Exclusion Criteria

We excluded non-Sudanese persons and patients had compromised disease.

2.6. Blood sample collection

A total of 3 ml of peripheral blood samples were collected from 100 patients diagnosed of leukemia and 100 non leukemic individual in EDTA tubes and stored at 4°C till Buffy coat formed. DNA extracted from EDTA blood samples by chelex 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 an agarose gel electrophoresis wild type, heterozygous and mutant genotypes for CYP3A5 and SULT1A1 were visualized on UV transeliminator.

2.7. Gene and Primer

Table (2.1)

Gene	**Ref Seq**	**Exon**	**Primer sequence**	
CYP3A5	*AC 005020*	3	*CATCAGTTAGTAGACAGATGA*	F
			GGTCCAAACAGGGAAGAAATA	*R*
SULT1A1	*KR709434.1*	7	*GTTGCCTCTGCAGGGTCTGGAGAGGAG*	F
			CCCAAACCCCCGTACTGGCCAGCACCC	R

2.8. DNA extraction methods

A total of 200 µL of a blood was placed into 1.5 eppendorf tube that contains 1 ml red blood cells lysis buffer, and was shacked occasionally for few minutes then incubated at room temperature ($37c^{o}$) for 30 minutes, centrifuged at 3500 rpm for 5 minutes, after the centrifugation the supernatant was discard and the process was repeated till white pellet was formed after that, 1 ml of white blood cell lysis buffer was added, shacked and incubated at RT for 15 minutes, centrifuged at 3500 rpm and the supernatant was then removed. And 200 µL of Insta Gene matrix (Chelex) was added to the remaining white pellet that was vortexed and incubated 56°C for 15 mines, then at 100 °C for 8 minutes before being Centrifuged at 10.000 rpm for 3 minutes after the centrifugation the supernatant was placed in eppendorftube.DNA concentration and purity was measured by Gene Quant machine and stored at-20°C for PCR analysis (Christopher *et al.*, 1996).

2.9. DNA Assessment

In a 1.5 ml separate eppendorf tube $10\mu l$ DNA was added to $90\mu l$ H_20 (1/10/dilution) was mixed and vortexes, then the mixture incubated for 10 min at RT to insure complete diffusion of DNA throughout the solution, an equal dilution of buffer TB Buffer was used as a blank, then DNA concentration ratio obtained at A260/280, Ideally the reading should be between 0.2 and 1

(Gene Quant, Amher shan, bioscience, UK).

2.10. Primer preparation

Each of the upstream and downstream primers were prepared by adding 10µl of each stock primer (100 µM) to 90µl distilled water and liquated

in 0.5 ml ependorffe tube to yield a concentration of 10µM, and the solution was mixed carefully using sterile tips to ensure the homogeneity.

2.11. Master Mix preparation

Samples and reagents were brought out from the freezer and kept on ice in a frozen cryo-rack during assembly procedure. A4 worksheet with PCR samples data was recorded for each sample to be tested. Master mix (MM) was prepared using forward and reverse primers for CYP3A5 and SULT1A1 into separate tube for each gene, the amount of each reagent was calculated and put into 1.5 µl sterile tube, according to the number of samples to be processed with an extra one more samples than actually being tested to compensate for retention of solution in pipette tips and tube. PCR reagents, except for samples DNA, were added in the order listed on the worksheet, adding water first and Taq polymerase last. The specified volume of MM was added into the each tube, all reagents were kept in a frozen-cryo-rack during mixing and returned to the freezer immediately after use, caps were closed tightly and the PCR tubes were moved to samples loading area. In the samples preparation area specified volume of sample was loaded into an appropriately labeled ready mix tube (Maxime PCR premix Kit). To avoid contamination, the tips were always changed and the avoidance of touching the side tube and capped was recommended.

2.12. Amplification of CYP3A5 gene

five μl of genomic *DNA* was added into ready prepared master mix for amplification of CYP3A5 gene for the Temperature profile as follows: initial denaturation 7 min at 94 °C, followed by 35 cycles of for (1 min at 94°C denaturation, 1 min at 55 °C Annealing, and 1 min at 72 °C extension) and finally elongated for 7 min at 72°C. 15 µl of the digested products were run on 2% agarose gel dissolved in 1X TBE buffer,

eletropherosed for 1 hour at 80v, and the fragments were visualized on UV trans eliminator (Bio RAD,USA).

2.13. Genotyping of CYP3A5 Variants Alleles

Genotyping of CYP3A5variants alleles by restriction fragment length. Ten microliter of the amplified products of CYP3A5 gene were digested with 5Units of Ssp1 enzyme in a total volume of twenty microliter mixture, Then incubated at 37°C over night.15 µl of the digested products were run on 2% agarose gel dissolved in 1X TBE buffer, eletropheroses for 1 hour at 80v, and the fragments were visualized on UV transellimantor,

2.14. Amplification of (SULT1A1) gene

5 µl of genomic DNA was added into ready prepared master mix for amplification of SULT1A1 gene for the Temperature profile as follows: initial denaturation 3 min at 94 ℃; 35 cycles of 1 min at 94°C denaturation, 1 min at 63 ℃ Annealing, and 1 min at 72 ℃ extension.

2.15. Polymorphism of (SULT1A1) gene

Ten microliter of the amplified products of SLUT 1A1 gene were digested with 5Units of Hae 11 enzyme in a total volume of twenty microliter mixture, incubated at 37°Cover night. 15 µl of the digested products were run on 2% agarose gel dissolved in 1X TBE buffer, eletropherosed for 1 hour at 80v, and the fragments were visualized on UV trans eliminator (Bio RAD, USA).

2.16. Statistical analysis

Data were entered and analyzed by SPSS program (version: 17.0). All demographic data of the study population were presented as mean ± SD. Data were analyzed using the Chi-square test for comparison the

prevalence of CYP3A5 and SULT 1A1 gene mutation between patients and controls (The test considered significant when (P. value <0.05), and the t-test was used for calculation the mean of hematological profile between the patients and controls.

2.17. Ethical approval

This study was approved by the medical laboratory college committee – SUST. Written consent was obtained from the participants after they had been informed with the study objectives, benefits and expected outcome. The participants were assured that the collected information will be kept confidential and will not be used for any other purpose other than this study. The laboratory tests were conducted in authorized to perform the study tests.

Chapter three

Results

Chapter three

Results

3.1. Results

Frequency of gender group shows leukemia patients male 62% female 38% and control group (male 82% and female 18%) (Fig.1).

Ethnic Distribution of the patients and controls show three groups, 73% were from Afro Asiatic (AA), 17% were from Nilo - Saharan (NS) and 10% were from Niger-Kongo (NK) in the patients, while in the control group 78% were from AA,14% were from NS and 8% were from NK in the control group **(**Fig 2**).**

The majority of the patients whom had CML, CLL, AML and ALL were from Afro Asiatic (AA), Nilo Saharan (NS) and Niger-Kongo (NK) had lower frequency (Table 1).

Related to gender type and occurrence of leukemia male were show to have a higher frequency of leukemia than females **(**Table 2).

The origin CYP3A5gene 293 bp digested and yield wild type (148bp, 125bp, and 20bp), mutant type (168bp, 125bp) or heterozygous (168bp, 148bp, 125bp, 20bp). (Bio RAD, USA). CYP3A5 genotypes show wild type allele CYP3A5*1/*1, heterozygous allele CYP3A5*1/*3 and mutant allele CYP3A5*3/*3 patients and controls (Table 3).

The origin of SULT1A1 gene 333bp digested and yield wild type 168mutant type165 or heterozygous (168bp + 165bp). (SULT1A1) genotypes shows wild type allele SULT *1/*1, heterozygous allele SULT *1/*2 and mutant allele SULT *2/*2 in patients and controls (Table 4).

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%).

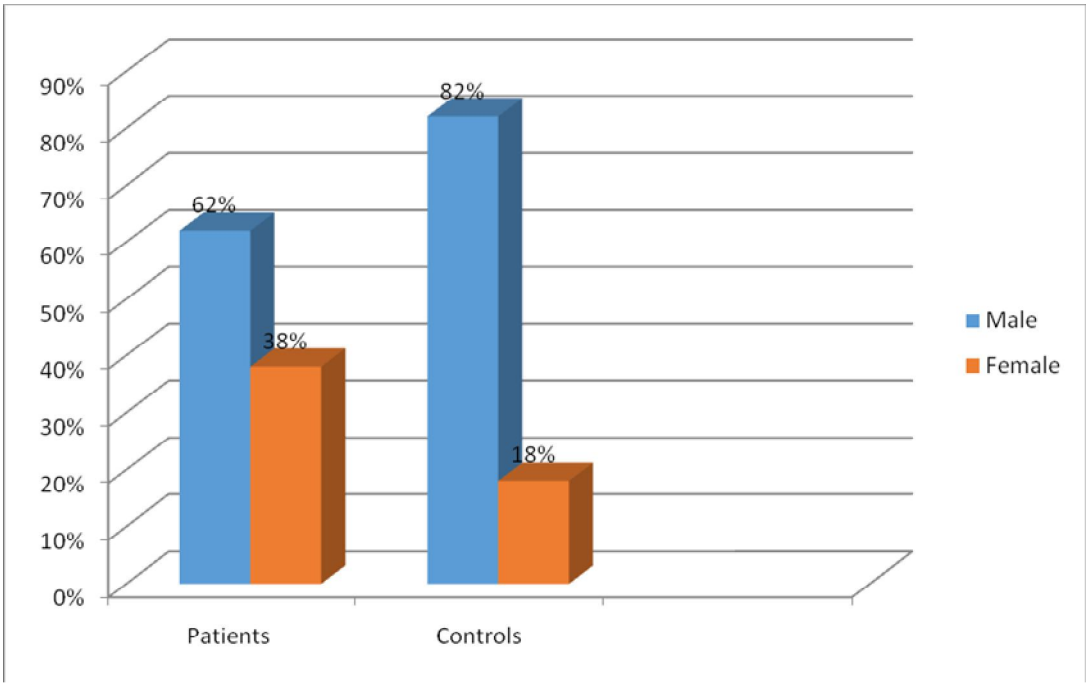

Fig. (1). Frequency of age and gender of the patients and controls

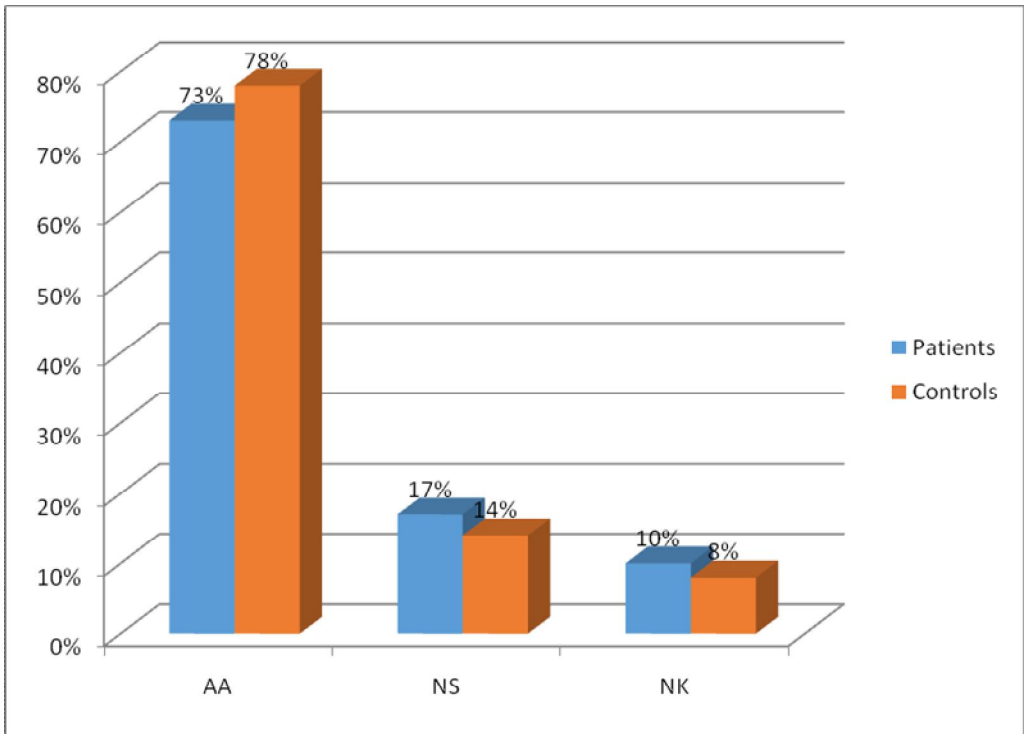

Fig. (3.2). Ethnic group distribution

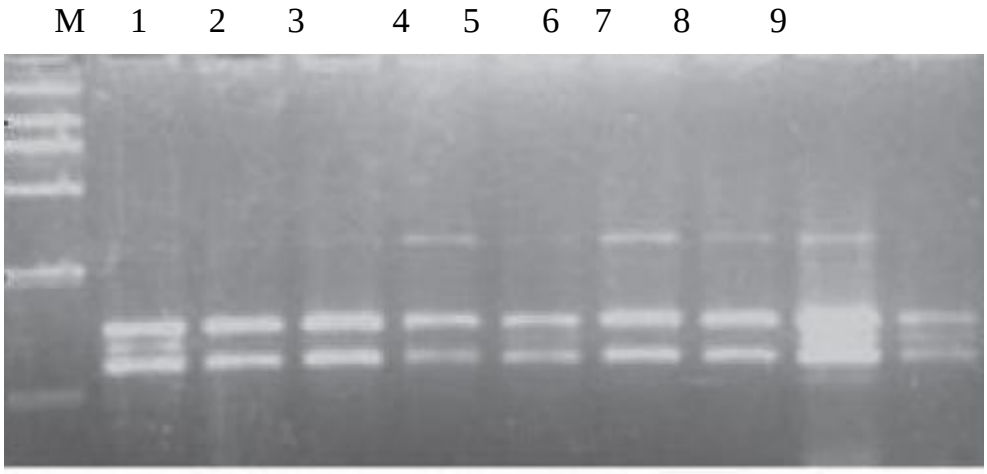

Fig. (3.3)CYP3A5PCR products after restriction digestion with Ssp1,Mdna marker 100 bp,lanes 1, 5, 8, 9 of AG genotype;lanes 2,4, 6, 7, of GG genotype

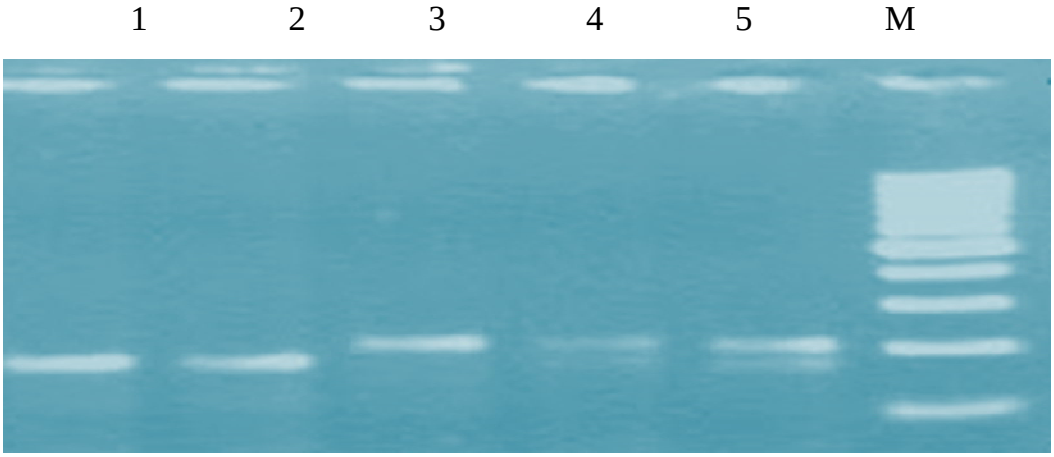

Fig. (3.4).SULT1A1 PCR products after restriction digestion with Hae ii on 2% agarose gel. M = 100bp DNA Ladder, Lane 1&2= GGgenotype, Lane 4&5 = GA genotype

Table(1). Ethnic and type of leukemia

Type of leukemia		CML	CLL	AML	ALL	Total
Ethnic	**AA**	19%	20%	14%	20%	73
	NS	4%	3%	5%	5%	17
	NK	2%	3%	2%	3%	10
Total		25%	26%	21%	28%	100

If;*P. value* ≤ 0.05 Chi-square=1.447, df=6 and *P.value*=0.963

Table (2). Gender and type of leukemia

Gender		Male	Female	Total
Type of leukemia	**CML**	16	9	25
	CLL	14	12	26
	AML	10	11	21
	ALL	22	6	28
Total		25%	62	38

Pvalue ≤ 0.05

Table (3). 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

Table (4). Frequency of SULT1A1Genotypes in Patientsandcontrols

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)

While the mutant SULT *2/*2 frequencies were 5(45.46%) in CML and 2 (18.18%) in CLL, AML and ALL respectively(Table 5 & 6).

The frequencies of mixed mutant genotype of CYP3 A5 and Sulphotransferes show that In CML 36.4%, CLL27.3%, AML 27.3 and ALL9.1% (Table, 7).

The frequency of CYP3A5 mutant alleles (CYP3A5*1/*3 and CYP3A5*3/*3) in patients with leukemia (Table 8).

The frequency of SULT1A1 mutant alleles (SULT1A1*1/*2 and SULT1A1*2/*2) in the mixed genotype (Table 9).

Table (5). 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 (6). 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

Table (7). Frequency of mixed mutant alleles of CYP3A5 and SULT1A1 in patients with leukemia

Type of leukemia	CYP3A5 and SULT1A1	Percentage%
CML	4	36.4
CLL	3	27.3
AML	3	27.3
ALL	1	9.0
Total	11	100

Table (8). Frequency of CYP3A5 mutant alleles (CYP3A5*1/*3 and CYP3A5*3/*3) in patients with leukemia

Type of leukemia	CYP3A5*1/*3	CYP3A5*3/*3	Total
CML	0	4	4
CLL	0	3	3
AML	3	0	3
ALL	0	1	1
Total	3	8	11

Table (9). Frequency of SULT1A1 mutant alleles (SULT1A1*1/*2 and SULT1A1*2/*2) in the mixed genotype

Typeof leukemia	SULT1A1*1/*2	SULT1A1*2/*2	Total
CML	0	4	4
CLL	1	2	3
AML	1	2	3
ALL	0	1	1
Total	2	9	11

Chapter four

Discussion, Conclusion and Recommendations

Chapter four

Discussion, Conclusion and Recommendations

4.1. 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. More occurrence of leukemic in male (62%) than in female (38%) of the population patients. Cancer mortality is higher among men than women (207.9 per 100,000 men and 145.4 per 100,000 women) because men are more exposure to carogenic effects than females. In this study we found that the frequency of leukemia are highest in Afro-Asiatic group males and lowest and other in ethnic groups as It is highest in African American men (261.5 per 100,000) and lowest in Asian/Pacific Islander women (91.2 per 100,000) (Ferlay J, 2012).Higher incidence of ALL in male gender. In contrast highest AML leukemia occurred in females. While chronic leukemia revealed higher incidence of CML and CLL in male patients than female. This findings is in agreement with other studies reported that there is much variability in incidence of leukemia in different populations(Au *et al*., 2009).CYP3A5 polymorphism in the present study determined three genotypes CYP3A5*1/*1 wild type, CYP3A5*1/*3heterozygous and significant increasesinCYP3A5*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 leukemia compared to those with acute leukemia. CYP3A5*3/*3 polymorphism explained significant elevation of mutant *3/*3genotype 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 CYP3A5expression associated with mutant

allele. Similar frequencies of CYP3A5*3/*3 allele in both the leukemic group and controls was reported by Liu (2002).On the other hand Shen *et al.*(2008) 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/*3 polymorphism with solid tumors in some populations like those of the Indian subcontinent, the frequencies of mutant CYP3A5*3/*3 alleles were elevated significantly in the CML group compared to controls (χ^2=93.15, df =2, p=0.0001) (Sailaja, 2010).Substantial raise of mutant CYP3A5*3/*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 (Bethke, 2007) 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/*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.,* 2007) mention that *SULT1A1* polymorphisms (phase II metabolism) modify the risk in ALL patients. Several studies have investigated the association of SULT1A1genes variants with cancer or leukemia, and the results have been controversial (Kotnis *et al*., 2008).

4.2. Conclusions

We concluded that 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.

4.3. Recommendations

1. Further analysis are recommended cover larger number of population to further confirm these results, which may provide a useful public health approach for early detection and prevention of leukemia.
2. Gender ages grouping is recommended to study leukemia under different age stages.
3. Regional ranking of leukemia are recommended to inspect the regional distribution of leukemia in different Sudanese states.
4. Furthers studies should be under taken to fully determine (CYP3A5) and (SULT1A1) contribution to pathogenesis of leukemia.

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Appendices

Appendix (i)

DNA Extraction Reagents and equipment

PCR Master Mix for PCR (TaqTM DNA polymearase + dNTPs +

Reaction buffer (10 xs).

PCR Master Preparation (H_2O 13 + Forward primer 1.0 + reverse primer 1.0 + master mix 5.0).

Heating Block dry Bath systems Taiwan

Bio Rad Thermal Cycler.Taiwan

Bio Rad KVM Incubator. Taiwan

Water bath.

Agarose gel.

Ethidium bromide.

Electrophoresis apparatous.

Gel Apparatus.

UV transeilliminater. UK

Automatic pipettes (20 µl, 100 µl, 5000 µl).

Sterile yellow and blue tips.

Appendix (ii) Heating Block dries Bath systems Taiwan

Appendix (iii) Gene Quantities- Amershan – Biosciences- U.K

Appendix (v) Centerfuge EPPENDROF - Germany -5430

Appendix (IV) FUME HOOD –Tel star- SPIN

Appendix (viii) Bio Rad Thermal Cycler.Taiwan

Appendix (x)

Questionnaire

Sudan University of Science and Technology

College of Graduates Studies

This questionnaire is on leukemia patients in RICK, Khartoum State.

Study introduced to obtain M.Sc. Degree.

Health center:……Date of the interview:……Patient Number ( ).

1. Personal character

Patient Name: ……Age:……. Gender: M( ) F( ) .

Residence:

……Tribe:……..……..Occupation:……

Proposed Insured please answer the following:

2. Type of Leukemia

ALL ( ) AML ( ) CLL( ) C ML( ).

3. Date of Diagnosis:

How was / is your Leukemia being treated (i.e. chemotherapy, bone marrow transplant, etc.)?.....

4. Date of last treatment: ……

Are you on any medications? Yes ( )No( ).

Is there a family history of cancer?Yes ( )No ( ) .

Date you last consulted your physician(s): ……..

Date:…….. Insured's Signature:…….

Appendix (ix)

جامعة السودان

كلية الدراسات العليا

دراسة لنيل درجة الماجستير

اقرار بالموافقة بالمشاركة

الاسم : .....

سوف يتم اخذ عينة من الدم الوريدي بواسطة حقنة , وذلك بعد تعقيم منطقة اخذ العينة بواسطة مطهر, جميع الأدوات المستخدمة معقمةومتبع فيها جميعوسائل السلامة المعملية وليست هنالك آثار جانبية للعملية,قديحدث تورم بسيط في منطقة اخذ العينة وسوف يزولهذاالتورمبعد فترة قصيرة . الغرض من اخذ هذه العينة هو البحث العلمي , وسوف يتم تسليمكم نسخة من النتائج , وسوف تحفظ النتائج بسرية تامة .

اوافق انا المذكور اعلاه علي اخذ عينة دم .

الاسم : .....التوقيع : .....