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Dedication

To My Family

Acknowledgements

First I thank Allah for giving me strength and help during my study.

I would like to thank my supervisor Prof. Babikar Ahmed Mohmmmed for his professional guidance and kind assistance which made this research came to light.

Also thank Dr. Hanan Babikar

I am also grateful to the staff and patients in RICK hospital

Abstract

This study was done in Radiation Iso top centre during the period 2011 to 2012 to assess the role of the Gene deletion of the detoxification enzymes glutathione S- transferees GSTMI and GSTTI in the occurrence of myeloid leukemia in Sudan. The study included 30 patients with acute myeloid leukemia, 30 patients with chronic leukemia and 30 healthy controls.

According to this study (46.7%) of the CML patients were from Khartoum State (where RICK) is located followed by the western Sudan in case of CML. (40%) of the AML patients were between the age of (40-50) years on the other hand most of the CML patients were between (40-50) the controls were between 31 - 40 years old.

The study revealed than both AML and CML patients were mostly from the Afro-asiatic population group, followed by Nile-Saharan in case of AML.

In this study the male / female ratio of AML was (1:2) (36.3% - 63.7%) while that of CML was (2:1) (66.7%-33.3%), the control group included males and females male / female ratio is (43.3%-56.7%).

Laboratory Methods:

- 1- Salting out method (DNA extraction).
- 2- Electrophoresis of extracted DNA.
- 3- Stander of PCR reaction (next Generation of premix (Germany CAPS. ALHP) master- mix.

Genotyping of patients showed that only 2 of the AML patients, 7 of the CML patients and 8 controls showed GSTMI while 22 of the AML patients 23 of the CML patients and 11 controls showed detected for the GSTTI.

For deletion in GSTM1 showed 6.67% of AML was deleted and, 23% of CML and 26.67% of controls were detected on the other hand for GSTTI and only 73% of AML 76.67% of CML and only 36.67% of controls were detected.

المستخلص

أجريت هذه الدراسة في مستشفى العلاج بالأشعة الطب النووي خلال الفترة بين 2011-2012 لتقييم دور الجينات الحذف إزالة السموم من الانزيمات الجلوتاثيون S- المنقولين GSTMI و GSTTI في حدوث سرطان الدم النخاعي في السودان. وشملت الدراسة 30 مريضاً يعانون من سرطان الدم النخاعي الحاد، سرطان الدم 30 مريضاً مع المزمن و 30 الاصحاء ظاهرياً.

وفقاً لهذه الدراسة معظم المرضى سرطان الدم الحاد وسرطان الدم المزمن من ولاية الخرطوم في مستشفى العلاج بالأشعة تليها المنطقة الغربية في حالة سرطان الدم المزمن وكان (46.7%) سرطان الدم الحاد (40%) تتراوح أعمارهم بين (40-50) سنة وسرطان الدم المزمن تتراوح أعمارهم بين (40-50) سنة، والاصحاء بين (31-40) سنة. الدراسة كشفت كلا من سرطان الدم الحاد والزمن المرضى هي في معظمها من مجموعة السكان الأفرو آسيوية، تليها القبائل النيلية في حالة سرطان الدم المزمن. في هذه الدراسة كانت نسبة الذكور إلى الإناث (1:2) في حالة سرطان الدم الحاد في حين أن سرطان الدم المزمن نسبة الذكور إلى الإناث كانت بنسبة (1:2) وشملت المجموعة الضابطة من الذكور والإناث. الاختبارات المعملية:

1- الحامض النووي DNA (طريقة إستخلاص الحامض النووي).

2- طريقة الكروماتوغرافي DNA.

3- طريقة الPCR.

أظهر التنميط الجيني للمرضى أن 2 فقط من المرضى سرطان الدم الحاد، و 7 من مرضى سرطان الدم المزمن و 8 مجموعة الإصحاء من GSTMI محذوفة في حين تم الكشف عن 22 من مرضى سرطان الدم الحاد و 23 من المرضى حالة سرطان الدم المزمن و 11 مجموعة الإصحاء. GSTTI محذوفة للكشف في GSTMI، تم الكشف عن 6.67% من سرطان الدم الحاد في حاله GSTMI، 23% من CML و 26.67% من الضوابط من جهة أخرى ل GSTTI و 73% فقط من AML من 76.67% CML وتم الكشف عن 36.67% فقط من مجموعة الإصحاء محذوفة.

List of Abbreviation

(APL)	Acute Promyelocytic leukemia
ALL	Acute lymphoblastic leukaemia
AmL	Acute myloid leukemia
AnLL	Acute Non lymphoblastic leukaemia
BM	Bone Marrow
(CBC)	Complete blood count
(CDNB)	Choro2,4- dinintrobenzene
(CGL)	Chronic granulocytic leukemia
CmL	Chronic myloid leukemia
(EFI)	Enzyme function initiative
(FAB)	French American British
GSTm₁	Glutathione S. Transferafes.m1
GSTT	Glutathione S. Transferafes.T1
(HTLV)	Human T-cell lymphotropic virus
Hcl	Hariy cell leukemia
(mPO)	Myeloperoidase
(NAP)	Neutrophils alkaline phosphates
(PB)	Peripheral blood
(PCR)	Polymerase Chain reaction
(Ph)	Philadephia chromosome
(S.BB)	Sudan black
(TKI_s)	Tyrosine kinase inhibitors
(T-PLL)	T- cell Prolymphocytic leukemia
RICK	Radiation Isotopes Center
(VNTR)	Variation in the number of tandem repeats
WHO	World Health Organization

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CHAPTER I

1. Introduction and Literature Reviews

1.1. Introduction:

Leukemia is a blood disorder caused by a primary neoplastic clonal disorder of the bone marrow (BM). A large number of neoplastic cells might be found in the peripheral blood with leukemic symptoms (Daenen, 1993) and (Bennett,1989).

Leukaemia is classified by the French American British Corporation (FAB). F- AB classification is used to distinguish between the main types of leukaemia and their sub-. Classifications (Bennett, 1989) and (Vaneys,1986). There are two different types of leukaemia, acute and chronic; these are further subdivided into myeloid and lymphoid (Valbuena ,2005). Acute leukaemia develops from the early blast cells and has two classes, Acute Lymphoblastic Leukaemia (ALL) and Acute Non lymphoblastic Leukaemia (ANLL); which are further subdivided according to the affected cell type (Valbuena,2005).

In chronic leukaemia the blood cells are more mature and divide at a slower rate. Chronic leukaemia takes two forms; Chronic Lymphocytic Leukaemia (CML) and Chronic Myelocytic Leukaemia(CML) (Bennett,1989). The former constitutes the subject of this project, as studies found that inter-individual differences in susceptibility to haematological malignancies may

be mediated in part through polymorphic variability in the bioactivation and detoxification of carcinogens (Cynara, 2008) and (Yuille, 2002). Accordingly, the glutathione transferases (GSTT1 and M1), methylenetetrahydrofolate reductase polymorphisms and cytochrome P450 3A5 1 *2C polymorphisms will be hypothesised and implicated acute myeloid leukaemia FAB classification of AML since 1976 the generally accepted.

WHO organization proposal of the classification of AML a clinical advisory committee of the (WHO) has proposed a new classification system for AML this is based not only on morphological appearances, but takes account of recurrent translocations, multilineage dysplasia, preceding haematological disorders and preceding chemo/radiotherapy.

Susceptibility genes in the current study for myeloid leukemia (CML and AML).

Classification of leukemia table (1.1) (Yuille, 2002).

Four major kinds of leukemia		
Cell type	Acute	Chronic
Lymphocytic leukemia (or "lymphoblastic")	Acute lymphoblastic leukemia (ALL)	Chronic lymphocytic leukemia (CLL)
Myelogenous leukemia (also "myeloid" or "nonlymphocytic")	Acute myelogenous leukemia (AML) (or myeloblastic)	Chronic myelogenous leukemia (CML)

1.2. Literature Review

1.2.1 Cancer in Africa:

The developing world, Africa included, is witnessing an alarming upsurge of cancer incidence. The annual number of new cancer cases is expected to double by 2020 and up to 70% of the 20 million new cases of cancer predicted to occur yearly will be in the developing world (Jones,1999) and (Ferlay, 2003) and (Yach, 2004). One startling disparity, however, between cancer in the developing and developed worlds is that although the overall incidence of cancer in the developing world is half of that observed in the developed world, survival rates in the developing world are often less than one third of site-specific cancers in the developed world (Sene,2005). This emphasizes the duality of the cancer problem in Africa, for being largely a disease of modern life style, occurring against a background of socio-economic disparities and greater burden of communicable diseases. The study of genetic epidemiology of cancers in Africa hence entails the study of peculiar features of gene-environment interaction that may be largely private to Africa. In addition to the state of socio-economic underdevelopment that applies almost to the majority of African states, there is the plethora of extreme environments, wide range of climatic conditions and cultures, but most of all the transition state of

the African communities from rural subsistent into urban market oriented life style.

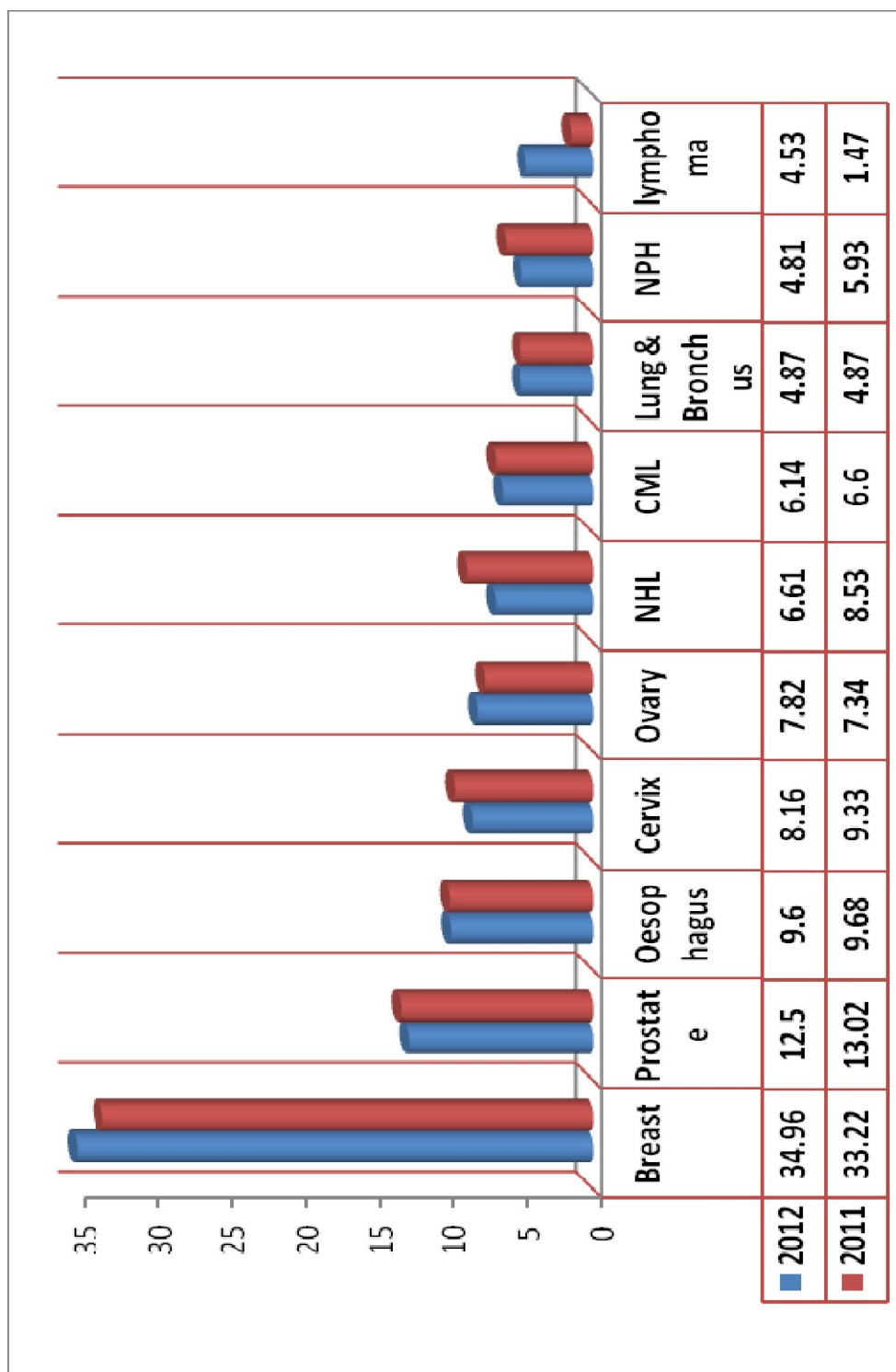
1.2.3. Cancer in Sudan Statistic Data:(statistic section in RICK)

1.2. Compared to more than 10 diseases reluctant 2011-2012

2011			2012		
Percent	No	Disease	Percent	No	Disease
33.22	1036	Breast	34.96	1242	Breast
13.02	406	Prostate	12.50	444	Prostate
9.68	302	Oesophagus	9.60	341	Oesophagus
9.33	291	Cervix	8.16	290	Cervix
7.34	229	Ovary	7.82	278	Ovary
8.53	266	NHL	6.61	236	NHL
6.60	206	CML	6.14	218	CML
4.87	152	Lung & Bronchus	4.87	173	Lung & Bronchus
5.93	185	NPH	4.81	171	NPH
1.47	46	lymphoma	4.53	161	lymphoma
47.04	3119	Total	49.73	3554	Total
52.96	3511	Residual	50.27	3592	Residual
100	6630	Total	100	7146	Total

Cancer in Sudan Statistic Data: (statistic section in RICK)

Compassion to more than 10 diseases reluctant 2011-2012



Clinically and pathologically, leukemia is subdivided into a variety of large groups. The first division is between its Acute and Chronic forms:

1.2.3.1. Acute leukemia: is characterized by a rapid increase in the number of immature blood cells. Crowding due to such cells makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children.

(Valbuena ,2005).

1.2.3.2.Chronic leukemia: is characterized by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal, resulting in many abnormal white blood cells. Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy. Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group.

Additionally, the diseases are subdivided according to which kind of blood cell is affected. This split divides leukemias into lymphoblastic or lymphocytic leukemias and myeloid or myelogenous leukemias:

- **In lymphoblastic** lymphocytic leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes, which are infection-fighting immune system cells. Most lymphocytic leukemias involve a specific subtype of lymphocyte, the B cell.
- **In myeloid** myelogenous leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form red blood cells, some other types of white cells, and platelets.

Combining these two classifications provides a total of four main categories. Within each of these four main categories, there are typically several subcategories. Finally, some rarer types are usually considered to be outside of this classification scheme.

- **Acute lymphoblastic leukemia** (ALL) is the most common type of leukemia in young children. This disease also affects adults, especially those age 65 and older. Standard treatments involve chemotherapy and radiotherapy. The survival rates vary by age: 85% in children and 50% in adults. (Valbuena,2005).

- Subtypes include precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia, and acute biphenotypic leukemia.
- **Chronic lymphocytic leukemia (CLL)** most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never affects children. Two-thirds of affected people are men. The five-year survival rate is 75%. (Thalhammer, 2002) It is incurable, but there are many effective treatments. One subtype is B-cell prolymphocytic leukemia, a more aggressive disease.
- **Acute myelogenous leukemia (AML)** occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. The five-year survival rate is 40%. (Cynara, 2008).
- Subtypes of AML include acute promyelocytic leukemia, acute myeloblastic leukemia, and acute megakaryoblastic leukemia.
- **Chronic myelogenous leukemia (CML)** occurs mainly in adults; a very small number of children also develop this disease. Treatment is with imatinib (Gleevec in US, Glivec in Europe) (Yuille, 2002) or other drugs. The five-year survival rate is 90%. (Fujihara, 2009) One subtype is chronic monocytic leukemia.
- **Hairy cell leukemia (HCL)** is sometimes considered a subset of chronic lymphocytic leukemia, but does not fit neatly into this

pattern. About 80% of affected people are adult men. No cases in children have been reported. HCL is incurable, but easily treatable. Survival is 96% to 100% at ten years. (Suzen, 2005) T-cell prolymphocytic leukemia (T-PLL) is a very rare and aggressive leukemia affecting adults; somewhat more men than women are diagnosed with this disease. (Taspinar M, Aydos SE, 2008) Despite its overall rarity, it is also the most common type of mature T cell leukemia; (Dufour, 2005) nearly all other leukemias involve B cells. It is difficult to treat, and the median survival is measured in months.

- **Large granular lymphocytic leukemia** may involve either T-cells or NK cells; like hairy cell leukemia, which involves solely B cells, it is a rare and indolent (not aggressive) leukemia. (Hung , 2003) and (Boffetta, 2005).
- **Adult T-cell leukemia** is caused by human T-lymphotropic virus (HTLV), a virus similar to HIV. Like HIV, HTLV infects CD4+ T-cells and replicates within them; however, unlike HIV, it does not destroy them. Instead, HTLV "immortalizes" the infected T-cells, giving them the ability to proliferate abnormally. Human T cell lymphotropic virus types I and II (HTLV-I/II) are endemic in certain areas of the world.

1.2.4. Myelogenous leukemia:

1.2.4.1. Chronic myelogenous leukemia

Chronic myelogenous (or myeloid) leukemia (CML), also known as chronic granulocytic leukemia (CGL), is a cancer of the white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors is the main finding. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome. CML is now largely treated with targeted drugs called tyrosine kinase inhibitors (TKIs), such as Gleevec/Glivec (imatinib), Sprycel (dasatinib), Tasigna (nilotinib), or Bosulif (bosutinib) which have led to dramatically improved long term survival rates (95.2%) since the introduction of Gleevec in 2001. These drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs.

1.2.4.2. Signs and symptoms

Patients are often asymptomatic at diagnosis, presenting incidentally with an elevated white blood cell count on a routine laboratory test. In this

setting, CML must be distinguished from a leukemoid reaction, which can have a similar appearance on a blood smear. Symptoms of CML may include enlarged spleen causing pain on the left side, malaise, joint and/or hip pain, low-grade fever, increased susceptibility to infections, anemia, and thrombocytopenia with easy bruising (although an increased platelet count (thrombocytosis) may also occur in CML). (Daenen S, 1993), and (Bennett,1989).

1.2.4.3. Diagnosis of CML

CML is often suspected on the basis on the complete blood count, which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased; this feature may help differentiate CML from a leukemoid reaction. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by detecting the Philadelphia chromosome. This characteristic chromosomal abnormality can be detected by routine cytogenetics, by fluorescent in situ hybridization, or by PCR for the bcr-abl fusion gene. (Bennett ,1989).

Controversy exists over so-called Ph-negative CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many patients in fact have complex chromosomal abnormalities that mask the

(9;22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. (Van Eys J, and Pullen J,(1986). The small subset of patients without detectable molecular evidence of bcr-abl fusion may be better classified as having an undifferentiated myelodysplastic/myeloproliferative disorder, as their clinical course tends to be different from patients with CML. (Valbuena ,2005).

1.2.4.4. Pathophysiology of CML

CML was the first malignancy to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania, USA: Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Center. (Thalhammer, 2002).

In this translocation, parts of two chromosomes (the 9th and 22nd by conventional karyotypic numbering) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. This abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight (p210 is short for 210 kDa protein, a shorthand used for characterizing proteins based solely on size).

Because carries a domain that can add phosphate groups to tyrosine residues (a tyrosine kinase), the bcr-abl fusion gene product is also a tyrosine kinase. (Cynara, 2008).

The fused BCR-ABL protein interacts with the interleukin 3beta(c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities. The action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia. With improved understanding of the nature of the BCR-ABL protein and its action as a tyrosine kinase, targeted therapies (the first of which was imatinib mesylate) that specifically inhibit the activity of the BCR-ABL protein have been developed. These tyrosine kinase inhibitors can induce complete remissions in CML, confirming the central importance of bcr-abl as the cause of CML. (Cynara CLS ,2008).

1.2.4.4. Classification of CML

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in

the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia. Drug treatment will usually stop this progression if started early. One of the drivers of the progression from chronic phase through acceleration and blast crisis is the acquisition of new chromosomal abnormalities (in addition to the Philadelphia chromosome). (Daenen,1993) Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed. (Bennett ,1989).

Chronic phase of CML

Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis. During this phase, patients are usually asymptomatic or have only mild symptoms of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase. (Bennett,1989).

Accelerated phase

Criteria for diagnosing transition into the accelerated phase are somewhat variable; the most widely used criteria are those put forward by

investigators at Anderson Cancer Center, (Yuille,2002), and (Sokal,1991) and the World Health Organization. The WHO criteria are perhaps most widely used, and define the accelerated phase by any of the following:

- 10–19% myeloblasts in the blood or bone marrow
- >20% basophils in the blood or bone marrow
- Platelet count <100,000, unrelated to therapy
- Platelet count >1,000,000, unresponsive to therapy
- Cytogenetic evolution with new abnormalities in addition to the Philadelphia chromosome
- Increasing splenomegaly or white blood cell count, unresponsive to therapy

The patient is considered to be in the accelerated phase if any of the above are present. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages. (Valbuena, 2005).

Blast crisis

Blast crisis is the final phase in the evolution of CML, and behaves like an acute leukemia, with rapid progression and short survival. Blast crisis

is diagnosed if any of the following are present in a patient with CML, (Suzen, 2004).

- >20% myeloblasts or lymphoblasts in the blood or bone marrow
- Large clusters of blasts in the bone marrow on biopsy
- Development of a chloroma (solid focus of leukemia outside the bone marrow)

1.2.4.5. Treatment of CML

Chronic phase: Chronic phase CML is treated with inhibitors of tyrosine kinase, the first of which was imatinib mesylate (marketed as Gleevec or Glivec; previously known as STI-571 or CGP57148B) approved by the United States FDA in 2001. Imatinib has proven to inhibit the progression of CML, allowing the majority of patients (65–75%) to have a regrowth of their normal bone marrow (cytogenetic response). Although few leukemic cells persist in most patients as evaluated by RT-PCR, and treatment has to be continued indefinitely, CML is the first cancer in which a medical treatment (imatinib) can give to the treated patients a normal life expectancy. (Taspinar, 2008).

To overcome imatinib resistance and to increase responsiveness of TK inhibitors, two novel agents were later developed. The first, dasatinib, is a TK inhibitor that blocks several oncogenic proteins and was initially

approved by the US FDA in 2007 to treat CML patients who were either resistant to or intolerant of imatinib. Another TK inhibitor, nilotinib, was also approved by the US FDA for the same indication. Nilotinib and dasatinib were approved for first-line therapy in 2010, so there are now three drugs available for first-line treatment of CML. In the past, antimetabolites (e.g., cytarabine, hydroxyurea), alkylating agents, interferon alfa 2b, and steroids were used, but these drugs have been replaced by TK inhibitor drugs. The TK inhibitor drugs specifically target BCR/abl, the constitutively activated tyrosine kinase fusion protein caused by the Philadelphia chromosome translocation. The IRIS study is an international study that compared interferon/cytarabine combination with imatinib. Long-term follow up demonstrating the superiority of imatinib regimens is clear-cut. Bone marrow transplantation was also used as initial treatment for CML before the advent of imatinib, but is now rarely used, primarily for those who fail drug treatment.

1.2.4.6. Prognosis of CML

A follow-up on patients using imatinib published in the New England Journal of Medicine in 2006 showed an overall survival rate of 89% after five years. (Barahmani ,2009).

In 2011, an independent study performed in 832 CML patients worldwide reported that the group of patients who achieve a stable cytogenetic response with imatinib shows an overall survival rate of 95.2% after 8 years, which is similar to the rate in the general population. Only 1% of patients died because of leukemia progression. (Taspinar, 2005).

1.2.4.7. Epidemiology of CML

CML occurs in all age groups, but most commonly in the middle-aged and elderly. Its annual incidence is 1–2 per 100,000 people, and slightly more men than women are affected. CML represents about 15–20% of all cases of adult leukemia in Western populations. (Daenen, 1993).

The only well-described risk factor for CML is exposure to ionizing radiation; for example, increased rates of CML were seen in people exposed to the atomic bombings of Hiroshima and Nagasaki (Robien ,2003).

Leukemia is also rarely associated with pregnancy, affecting only about 1 in 10,000 pregnant women. (Joseph,2004) Chronic myelogenous leukemia can be treated with relative safety at any time during pregnancy with Interferon-alpha hormones

1.2.5. Acute myeloid leukemia

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia, is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. Although AML is a relatively rare disease, accounting for approximately 1.2% of cancer deaths in the United States, its incidence is expected to increase as the population ages (Taspinar, 2005).

The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, which causes a drop in red blood cells, platelets, and normal white blood cells. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. Several risk factors and chromosomal abnormalities have been identified, but the specific cause is not clear. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated (Taspinar, 2005).

AML has several subtypes; treatment and prognosis varies among subtypes. Five-year survival varies from 15–70%, and relapse rate varies

from 33–78%, depending on subtype. AML is treated initially with chemotherapy aimed at inducing a remission; patients may go on to receive additional chemotherapy or a hematopoietic stem cell transplant. Recent research into the genetics of AML has resulted in the availability of tests that can predict which drug or drugs may work best for a particular patient, as well as how long that patient is likely to survive (Barahmani ,2009).

1.2.5.1. Signs and symptoms of AML

Most signs and symptoms of AML are caused by the replacement of normal blood cells with leukemic cells. A lack of normal white blood cell production makes the patient susceptible to infections; while the leukemic cells themselves are derived from white blood cell precursors, they have no infection-fighting capacity. (Cynara, 2008).

A drop in red blood cell count (anemia) can cause fatigue, paleness, and shortness of breath. A lack of platelets can lead to easy bruising or bleeding with minor trauma.

The early signs of AML are often vague and nonspecific, and may be similar to those of influenza or other common illnesses. Some generalized symptoms include fever, fatigue, weight loss or loss of appetite, shortness of breath, anemia, easy bruising or bleeding, petechiae (flat, pin-head

sized spots under the skin caused by bleeding), bone and joint pain, and persistent or frequent infections. (Cynara, 2008).

Enlargement of the spleen may occur in AML, but it is typically mild and asymptomatic. Lymph node swelling is rare in AML, in contrast to acute lymphoblastic leukemia. The skin is involved about 10% of the time in the form of leukemia cutis. Rarely, Sweet's syndrome, a paraneoplastic inflammation of the skin, can occur with AML. (Cynara,2008).

Some patients with AML may experience swelling of the gums because of infiltration of leukemic cells into the gum tissue. Rarely, the first sign of leukemia may be the development of a solid leukemic mass or tumor outside of the bone marrow, called a chloroma. Occasionally, a person may show no symptoms, and the leukemia may be discovered incidentally during a routine blood test. (Yuille, 2002).

1.2.5.2. Diagnosis of AML

The first clue to a diagnosis of AML is typically an abnormal result on a complete blood count. While an excess of abnormal white blood cells (leukocytosis) is a common finding, and leukemic blasts are sometimes seen, AML can also present with isolated decreases in platelets, red blood cells, or even with a low white blood cell count (leukopenia). (Duthie,1997).

While a presumptive diagnosis of AML can be made via examination of the peripheral blood smear when there are circulating leukemic blasts, a definitive diagnosis usually requires an adequate bone marrow aspiration and biopsy.

Marrow or blood is examined via light microscopy, as well as flow cytometry, to diagnose the presence of leukemia, to differentiate AML from other types of leukemia (e.g. acute lymphoblastic leukemia - ALL), and to classify the subtype of disease. A sample of marrow or blood is typically also tested for chromosomal abnormalities by routine cytogenetics or fluorescent in situ hybridization. Genetic studies may also be performed to look for specific mutations in genes such as FLT3, nucleophosmin, and KIT, which may influence the outcome of the disease. (Duthie,1999).

Cytochemical stains on blood and bone marrow smears are helpful in the distinction of AML from ALL, and in subclassification of AML. The combination of a myeloperoxidase or Sudan black stain and a nonspecific esterase stain will provide the desired information in most cases. The myeloperoxidase or Sudan black reactions are most useful in establishing the identity of AML and distinguishing it from ALL. The nonspecific esterase stain is used to identify a monocytic component in AMLs

and to distinguish a poorly differentiated monoblastic leukemia from ALL.

The diagnosis and classification of AML can be challenging, and should be performed by a qualified hematopathologist or hematologist. In straightforward cases, the presence of certain morphologic features (such as Auer rods) or specific flow cytometry results can distinguish AML from other leukemias; however, in the absence of such features, diagnosis may be more difficult. (Blount,1997).

According to the widely used WHO criteria, the diagnosis of AML is established by demonstrating involvement of more than 20% of the blood and/or bone marrow by leukemic myeloblasts. (Ski ,1999).

The French–American–British (FAB) classification is a bit more stringent, requiring a blast percentage of at least 30% in bone marrow (BM) or peripheral blood (PB) for the diagnosis of AML. (Wiemels,2001). AML must be carefully differentiated from "preleukemic" conditions such as myelodysplastic or myeloproliferative syndromes, which are treated differently.

Because acute promyelocytic leukemia (APL) has the highest curability and requires a unique form of treatment, it is important to quickly establish or exclude the diagnosis of this subtype of leukemia. Fluorescent in situ

hybridization performed on blood or bone marrow is often used for this purpose, as it readily identifies the chromosomal translocation that characterizes APL. (Bolufer,2007).

1.2.5.3. Pathophysiology of AML

The malignant cell in AML is the myeloblast. In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells; a normal myeloblast will gradually mature into a mature white blood cell. In AML, though, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation. (Moon,2007). Such a mutation alone does not cause leukemia; however, when such a "differentiation arrest" is combined with other mutations which disrupt genes controlling proliferation, the result is the uncontrolled growth of an immature clone of cells, leading to the clinical entity of AML. (Genoud, 2000).

Much of the diversity and heterogeneity of AML stems is because leukemic transformation can occur at a number of different steps along the differentiation pathway. (Guenther,1999) Modern classification schemes for AML recognize the characteristics and behavior of the leukemic cell (and the leukemia) may depend on the stage at which differentiation was halted.

Specific cytogenetic abnormalities can be found in many patients with AML; the types of chromosomal abnormalities often have prognostic significance. (Frosst,1995).

The chromosomal translocations encode abnormal fusion proteins, usually transcription factors whose altered properties may cause the "differentiation arrest". (Kluijtmans,1997). For example, in acute promyelocytic leukemia, that (Barahmani, 2009) and (Kusumakumary,2004), translocation produces a PML-RAR α fusion protein which binds to the retinoic acid receptor element in the promoters of several myeloid-specific genes and inhibits myeloid differentiation. (Lovricevic and, Franjic, 2004).

The clinical signs and symptoms of AML result from the growth of leukemic clone cells, which tends to displace or interfere with the development of normal blood cells in the bone marrow. (*Morita, Taguchi et al*, 1997). This leads to neutropenia, anemia, and thrombocytopenia. The symptoms of AML are, in turn, often due to the low numbers of these normal blood elements. In rare cases, patients can develop a chloroma, or solid tumor of leukemic cells outside the bone marrow, which can cause various symptoms depending on its location. (Cynara, 2008).

1.2.5.4. Treatment of AML

First-line treatment of AML consists primarily of chemotherapy, and is divided into two phases: induction and postremission (or consolidation) therapy. The goal of induction therapy is to achieve a complete remission by reducing the number of leukemic cells to an undetectable level; the goal of consolidation therapy is to eliminate any residual undetectable disease and achieve a cure. (Stevenson, 1996).

Hematopoietic stem cell transplantation is usually considered if induction chemotherapy fails or after a patient relapses, although transplantation is also sometimes used as front-line therapy for patients with high-risk disease.

1.2.6. Risk factors of leukemia:

1.2.6.1 Environmental and nutrition factors:

factors rather than genetic factors are the key determinants of the international variation in cancer rates. As far as Africa is concerned, African Americans' disease data, represent a working model to test the role of changing environment and the effect of life style in complex diseases. Interestingly both sides of the argument seem to find support. Chronic myeloid leukemia (CML) patients show worse survival for African American and Hispanics compared to Americans of European origin (Lee,2009). Although the difference in

ethnicity data might be argued to reflect socio-economic differences, the current advances in genomics enable the implication of particular genomic regions and genes that explain the ethnic differences in susceptibility to infectious and chronic diseases.

Nutritional factors have also been implicated, adding an extra layer of complexity to the desperately compound picture of cancer etiology. Data on nutrition is greatly deficient in Africa similar to other aspects of genetic epidemiology, although differences in nutritional practices and culture may be key in providing vital clues to the contribution of life style. A study in China Zhang, (2008), for example, suggested that a higher intake of green tea is associated with a reduced risk of adult leukemia.

AMLrisk was negatively associated with milk intake among women and tea, and positively associated among women with beer, wine and beef (Li, 2006).

A prospective cohort

study by (Ma et al,2009) showed that smoking and total meat intake were risk factors for AML and those who did not drink coffee appeared to have a higher risk of AML.

1.2.6.1. Causes of AML

A number of risk factors for developing AML have been identified, including: other blood disorders, chemical exposures, ionizing radiation, and genetics.

1.2.6.2. Preleukemia

"Preleukemic" blood disorders, such as myelodysplastic syndrome or myeloproliferative disease, can evolve into AML; the exact risk depends on the type of MDS/MPS. (Pearson and Soli, 1991).

1.2.6.3. Chemical exposure

Exposure to anticancer chemotherapy, in particular alkylating agents, can increase the risk of subsequently developing AML. The risk is highest about three to five years after chemotherapy. (Fujihara, 2009). Other chemotherapy agents, specifically epipodophyllotoxins and anthracyclines, have also been associated with treatment-related leukemia. These treatment-related leukemias are often associated with specific chromosomal abnormalities in the leukemic cells. (Ada , 2004).

Occupational chemical exposure to benzene and other aromatic organic solvents is controversial as a cause of AML. Benzene and many of its derivatives are known to be carcinogenic in vitro. While some studies have suggested a link between occupational exposure to benzene and increased risk of AML, (Taspinar, 2008). Others have suggested the attributable risk, if any, is slight. (Dufour, 2005).

1.2.6.4. Radiation

Ionizing radiation exposure can increase the risk of AML. Survivors of the atomic bombings of Hiroshima and Nagasaki had an increased rate of AML, (Hung, 2003). As did radiologists exposed to high levels of X-rays prior to the adoption of modern radiation safety practices. (Olshan, 2000).

1.2.6.5. Genetics

A hereditary risk for AML appears to exist. Multiple cases of AML developing in a family at a rate higher than predicted by chance alone have been reported. (Barahmani, 2009) ; (Robien, 2003) ; (Joseph, 2004) (Krajinovic, 2002). The risk of developing AML is increased threefold in first-degree relatives of patients with AML.

Several congenital conditions may increase the risk of leukemia; the most common is probably Down syndrome, which is associated with a 10- to 18-fold increase in the risk (Chang, 2003).

1.2.7. Incidence and epidemiology

The lowest rates of leukemia reported in sub-Saharan Africa probably represent failure of diagnosis or reporting to some extent (Parkin, 2005). We should therefore use caution, when drawing conclusions based on the varying prevalence and incidence, as an indication of clustering of cases or an environmental or genetic effect, as this may simply be due to the

deficiency of statistics in Africa. The disparity could also be a reflection of the research milieu and capacity of individual countries or research groups, which indeed seem to be the case as most of the current reports on leukemia emerges from countries with well established science capacities. Even with the scattered and available data, however, the difference from European and global trends could be observed as well as the evolution of the problem of leukemia. An early report from Uganda that African children in Uganda showed a great and genuine deficiency of leukemia and an excess of solid lympho-reticular tumors (Davies, 1965). In subsequence, the situation seems to change, a study of pediatric leukemia in Cameroon, showed that Acute Lymphoblastic Leukemia (ALL) comprised 78.6%, while AML 21.4% of all pediatric acute leukemia (Obama, 1995). In Egypt, the lymphatic and haemopoietic cancer incidence in 2001 have increased approximately 11-fold compared with the incidence in 1972. Moreover, the incidence of leukemia among infant less than 5 years increased exponentially with a higher incidence among boys (Hosny,2002). In Kenya, leukemia in children below the age of 15 years comprised 37% of leukemia in all ages. Childhood acute leukemia formed 52.3% of all the acute leukemia. AML and ALL occurred, in almost equal proportions 42 % and 46 % respectively (Kasili,1990). In Ethiopia, a report by (Shamebo, 1990) showed that the commonest type of leukemia was CML 57.8%, acute leukemia and chronic lymphatic leukemia (CLL) accounted for 21.1% each. Of the acute

leukemia, 53.3% were ALL while 46.7% were AML (Shamebo,1990). A recent study in south Nigeria showed that AML comprises 12.3% and CML 23.9% of all leukemia, with a mean age at diagnosis of 25.6 years and 35.2 years respectively (Nwannadi,2011). In the last 25 years in Sudan CML became the predominant cancer in men, while lymphomas remained the second most common cancer. In women, breast, cervical and ovarian cancer remained the three most common cancers over both time periods, but there was also an increase in the incidence of CML among women (Hamad,2006), the causes of this high incidence of CML are not known. In Europe AML presents as mainly an adult's disease with a median age at presentation of 64 years, accounting for around 30% of all leukemia in adults, and ~18 000 new patients are diagnosed in Europe each year, representing ~0.6% of all cancers. The annual incidence rate in Europe ranges from two per 100 000/year to four per 100 000/year. In the past decade, the trend in overall incidence of AML has generally been stable or slowly increasing in most European countries, while most cases of CML occur in adults with a median age at presentation around age 60. CML comprises only around 2%–3% of all the leukemia diagnosed in patients <20 years of age but the incidence increases with age slowly until the mid-40s, then more rapidly from about one per 1000 000/year in children <10 years to two per 100 000 in people in the fifth decade to one per 10 000 at age 80. The disease is more common in males. There is no clear evidence of to geographic or ethnic background that

predisposes to CML; however, in the United States the incidence is slightly higher in Caucasians than in Blacks or (Hispanics Lee,2009), and it exhibits a male preponderance, in South African Coloured and Black people, and African Americans in comparison to whites but the reason for this is unexplained.

Furthermore, several reports showed an increased incidence of CML during the first 2 decades of life in African subjects but other reports , disagree with this. [Jacobs et al. reported that in comparison with Whites and Coloureds, however, the peak incidence for Blacks was lower, lying between the 3rd and 4th decades. The peak incidence for Coloureds was in the 4th and 5th decades, and that for Whites in the 5th and 6th.

An interesting study in United Arab Emirate (UAE) found that the rate of AML among UAE female nationals was higher than in nationals male and expatriates. The study proposed that chemicals in henna dye, which is used to decorate the body, as well as a lack of sunlight could be behind the increased incidence (Hassan ,2009). Henna is applied in many African countries especially Sudan where it is used by vast majority of Sudanese married women.

1.2.7.1. Molecular etiology

Leukemia in common with other cancers arises from mutations in a single cell, which enable the cell to reproduce excessively, emerging as a dominant clone. A large number of different mutant genes contribute to

leukaemogenesis singly or, more often, in combination and many are leukemia sub-type specific. The number of genetic abnormalities is believed to reflect the number of genes that control distinct developmental stages of blood formation and the multiple routes to clonal dominance. These include changes not only in proliferative activity, but in ability to differentiate, in resistance to cell death, in DNA repair activity and in general stability of the genes. Whether any particular mutant genes, or hot spots' for mutations within a gene, are linked to particular DNA damaging agents is a topic of considerable relevance to the molecular epidemiology of cancer in general. For these changes to happen some culprit agents has to come into action namely ionizing radiation, chemicals such as polycyclic hydrocarbons and certain drugs, and viruses. The way how these extrinsic factors affect the cell genetically and epigenetically is the core of the functional research in cancer. Although multiple risk factors have been linked to the development of leukemia, however these known risk factors account for only a small number of observed cases. Few epidemiological studies have explored the relation between lifestyle, dietary factors and the incidence of adult Leukemia, and almost none has addressed the molecular and genetic aspects of these interactions (Barahmani, 2009).

- **Population and ethnic diversity**

In the last decade the importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis has been

realised. Gender and ethnic differences in these years should have a focus in health policy in Africa. A study by (Lee et, 2009) al. examined the demographic and clinical features of CML in an ethnically diverse population and found that Hispanic patients present with lower risk profile CML and achieve better treatment responses compared to non-Hispanic patients. The vast majority of their non-Hispanic patients were African American or Asian. This study proposed that biological/genetic factor can contribute to this observed ethnic differences in disease presentation and behavior. Hispanic ethnic group is thought to be the least diverse ethnic group, at the opposite site the African descent is the most diverse ethnic group. African populations are characterized by greater levels of genetic diversity, extensive population substructure compared to non-African moreover Africa shows a wide range of environments, climatic, vegetative and zoological. Thus human cancer patterns are expected to show a similar degree of diversity, the study of which would contribute to our understanding of their causes (Barahmani,2009).

1.2.7.2. Gene and chromosomal rearrangements

The capacity of genetic investigation in Africa of cancer susceptibility genes and chromosomal aberrations linked to cancer, makes the picture even more opaque. both molecular and genetic abnormalities became an important factor for characterising, treating and risk stratifying of myeloid leukemia. In 2002 the WHO classification of Myeloid Neoplasms showed that AML classification includes specific genetic subcategories; thus,

determination of genetic features of the neoplastic cells must be performed if possible. Many recurring genetic abnormalities in the myeloid neoplasms can be identified by advanced molecular and cytogenetic techniques. In addition, patients with the clonal, recurring cytogenetic abnormalities $t(8;21)(q22;q22)$, $inv(16)(p13q22)$ or $t(16;16)(p13;q22)$, and $t(15;17)(q22;q12)$ should be considered to have AML regardless of the blast percentage. On the other hand, according to the WHO classification, CML is defined specifically as a myeloproliferative disease that is characterized by the invariable presence of the Philadelphia (Ph) chromosome or the BCRABL fusion gene. Chromosomal translocations in myeloid leukemia yield hybrid RNAs capable of encoding fusion chimeric proteins. The unique amino acid sequences found in these oncogenic fusion proteins represent true tumor-specific antigens that are potentially immunogenic. Barahmani,(2009).

1.2.8 Xenobiotic metabolism

refers to the various chemical reactions, called metabolic pathways, which a living organism uses to alter chemicals that are not normally found in an organism as part of its natural biochemistry. These chemicals, called xenobiotics, can include things such as poisons, drugs, and environmental pollutants. Xenobiotic metabolism is important for life, as it allows an organism to neutralize and eliminate foreign toxins that would otherwise interfere with the chemical processes that keep it alive. The xenobiotic

metabolism of humans and many other forms of life is important in fields such as medicine, agriculture, and environmental science. Many potentially harmful substances are prevented from doing damage by the membranes of cells, which regulate which chemicals are allowed to enter a cell and physically block many xenobiotics. Polar molecules, which have electric dipoles because their electrons are not evenly shared among the molecule's atoms, are generally unable to get past a cell's membrane. Nonpolar molecules, however, can pass through the permeable membrane and into the cell. Xenobiotic metabolism protects the body from these substances with enzymes that will react with most nonpolar compounds. This specialization prevents them from attacking helpful substances that are part of the organism's normal biochemistry, which are polar compounds able to diffuse through cellular membranes with the aid of transport proteins. Xenobiotic metabolism is divided into three phases. In phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds in phase II reactions. These reactions are catalysed by transferase enzymes such as glutathione S-transferases. Finally, in phase III, the conjugated xenobiotics may be further processed, before being recognised by efflux transporters and pumped out of cells. The reactions in these pathways are of particular interest in medicine as part of drug metabolism and as a factor contributing to multidrug

resistance in infectious diseases and cancer chemotherapy. The actions of some drugs as substrates or inhibitors of enzymes involved in xenobiotic metabolism are a common reason for hazardous drug interactions. These pathways are also important in environmental science, with the xenobiotic metabolism of microorganisms determining whether a pollutant will be broken down during bioremediation, or persist in the environment. The enzymes of xenobiotic metabolism, particularly the glutathione S-transferases are also important in agriculture, since they may produce resistance to pesticides and herbicides.

1.2.8.1. Phases of detoxification.

The metabolism of xenobiotics is often divided into three phases: modification, conjugation, and excretion. These reactions act in concert to detoxify xenobiotics and remove them from cells.

1.2.8.2. Detoxification enzyme

Phase I: Cytochrome P450 3A5(CYP450) is a family of genes which have a role in the detoxification of the reactive oxygen species, chemotherapeutic agents and environmental carcinogens Chang BL, Zheng,(2003). CYP 3A5 gene catalyzes the bio-activation of polycyclic aromatic hydrocarbons and it is polymorphic, encoding the CYP 3A5 enzyme Chang BL, Zheng SL, (Isaacs SO,2003). CYP 3A5 gene polymorphisms have been widely studied, particularly in terms of cancer

susceptibility. CYP 3A5 * 2C polymorphism is one of the most eminent CYP IAJ gene polymorphisms. Its mutation is 2455A3G at the exon 7 region. This leads to IleVal replacement in the catalytic region of the CYP1A1 protein (Taspinar, 2008). The CYP 3A5 *2C polymorphism has been detected to varying degrees among different ethnic groups Taspinar,(2008). Higher levels of adduct formation arise from enzyme activity in IleVal and Val/Val variants of CYP1A1. This elevates the risk of cancers including myeloid leukemia (Saito,2003).

Existing findings have shown that CYP 3A5 and GST genes have numerous genetic polymorphisms. CYP 3A5 and GST polymorphisms were lacking protein functionality (Hung,2005), leading to either elevated or minimized metabolic activity (Hung,2003).

CYP 3A5 and GST polymorphisms may modify the metabolism of carcinogens and mutagens. This is a primary causation of cancer and it would be reasonable to assume a relationship with the polymorphism of genes encoding xenobiotic metabolism.

A study suggested that polymorphic CYP 3A5 and GSTT1, M1 genes emerge to influence the susceptibility to CML and AML Taspinar,(2008), of the CML and AML patients, 19.20/0 patients had CYP 3A5 Val allele, which indicates the presence of CML and AML. However, the frequency of GSTM I polymorphism was not statistically significant among those

patients (44.9%) in comparison with the controls (42.3%). In contrast, 40.2% of the CML and AML patients were found with a GSTTI polymorphism. This was significantly higher compared to the controls. This presence may be a protective factor in CML and AML patients.

Phase 11: Glutathione S-transferases (GSTT)

Affiliated to the enzyme cluster that is broadly expressed in mammalian tissues. These enzymes have wide substrate specificity and polymorphic genes e.g. GSTT and GSTM (Joseph,2004). by showed that polymorphisms in the GST family of enzymes have been associated with survival and occurrence of toxicity in children and adults who have leukaemia (Yuille,2002). It is therefore worth to know about these polymorphisms in Sudanese population with CML and AML, as a pattern study for the further future studies in other cancers. These genes play a main role in the metabolism of xenobiotics, counting chemotherapeutic agents, reactive species and environmental carcinogens. The role of GSTT and GSTM in cancer has already been well documented (Joseph, 2004) and (Krajinovic, 2002).

Phase I - modification

In phase I, a variety of enzymes acts to introduce reactive and polar groups into their substrates. One of the most common modifications is



hydroxylation catalysed by the cytochrome P-450-dependent mixed-function oxidase system. These enzyme complexes act to incorporate an atom of oxygen into nonactivated hydrocarbons, which can result in either the introduction of hydroxyl groups or N-, O- and S-dealkylation of substrates. The reaction mechanism of the P-450 oxidases proceeds through the reduction of cytochrome-bound oxygen and the generation of a highly-reactive oxyferryl species, according to the following scheme:

Phase II - conjugation

In subsequent phase II reactions, these activated xenobiotic metabolites are conjugated with charged species such as glutathione (GST), sulfate, glycine, or glucuronic acid. These reactions are catalysed by a large group of broad-specificity transferases, which in combination can metabolise almost any hydrophobic compound that contains nucleophilic or electrophilic groups. One of the most important of these groups are the glutathione S-transferases (GSTs). The addition of large anionic groups (such as GST) detoxifies reactive electrophiles and produces more polar metabolites that cannot diffuse across membranes, and may, therefore, be actively transported (Yuille, 2002).

Phase III - further modification and excretion

After phase II reactions, the xenobiotic conjugates may be further metabolised. A common example is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates. Here, the γ -glutamate and glycine residues in the glutathione molecule are removed by Gamma-glutamyl transpeptidase and dipeptidases. In the final step, the cystine residue in the conjugate is acetylated (Joseph, 2004).

Conjugates and their metabolites can be excreted from cells in phase III of their metabolism, with the anionic groups acting as affinity tags for a variety of membrane transporters of the multidrug resistance protein (MRP) family. These proteins are members of the family of ATP-binding cassette transporters and can catalyse the ATP-dependent transport of a huge variety of hydrophobic anions, and thus act to remove phase II products to the extracellular medium, where they may be further metabolised or excreted. (Ada *et al.*, 2004).

1.2.9. Endogenous toxins

The detoxification of endogenous reactive metabolites such as peroxides and reactive aldehydes often cannot be achieved by the system described above. This is the result of these species' being derived from normal cellular constituents and usually sharing their polar characteristics.

However, since these compounds are few in number, it is possible for enzymatic systems to utilize specific molecular recognition to recognize and remove them. The similarity of these molecules to useful metabolites therefore means that different detoxification enzymes are usually required for the metabolism of each group of endogenous toxins. Examples of these specific detoxification systems are the glyoxalase system, which acts to dispose of the reactive aldehyde methylglyoxal, and the various antioxidant systems that remove reactive oxygen species(Yuille,2002).

1.2.9.1. The glutathione S-transferase

The glutathione S-transferase (GST, previously known as ligandins) family of enzymes are composed of many cytosolic, mitochondrial, and microsomal (now designated as MAPEG) proteins. GSTs are present in eukaryotes and in prokaryotes, where they catalyze a variety of reactions and accept endogenous and xenobiotic substrates. Members of the GST superfamily are extremely diverse in amino acid sequence, and a large fraction of the sequences deposited in public databases are of unknown function. The Enzyme Function Initiative (EFI) is using GSTs as a model superfamily to identify new GST functions (Ada and Suzen, 2004).

GSTs can constitute up to 10% of cytosolic protein in some mammalian organs. GSTs catalyse the conjugation of reduced glutathione — via a

sulfhydryl group — to electrophilic centers on a wide variety of substrates. This activity detoxifies endogenous compounds such as peroxidised lipids, as well as breakdown of xenobiotics. GSTs may also bind toxins and function as transport proteins, which gave rise to the early term for GSTs of “ligandin”. The mammalian GST super-family consists of cytosolic dimeric isoenzymes of 45–55 kDa size that have been assigned to at least six classes: Alpha, Mu, Pi, Theta, Zeta and Omega. (Ada and Suzen, 2004).

Most mammalian isoenzymes have affinity for the substrate 1-chloro-2,4-dinitrobenzene (CDNB), and spectrophotometric assays utilising this substrate are commonly used to report GST activity. (Taspinar and Aydos, 2008). However, some endogenous compounds, e.g., bilirubin, can inhibit the activity of GSTs. In mammals, GST isoforms have cell specific distributions (e.g., alpha GST in hepatocytes and pi GST in the biliary tract of the human liver). (Dufour and Svahn, 2005).

1.2.9.2. Naming and classification of GSTs:

The glutathione *S*-transferase (GST) family of soluble, dimeric enzymes catalyzes the conjugation of glutathione with a wide variety of electrophiles, generally resulting in detoxification and facilitated elimination.

Table (1.3): Classes of Human GST Gene

Class	Members
Alpha	GSTA1, GSTA2, GSTA3, GSTA4, GSTA5
Kappa	GSTK1
Mu	GSTM1, GSTM1L, GSTM2, GSTM3, GSTM4, GSTM5
Omega	GSTO1, GSTO2
Pi	GSTP1
Theta	GSTT1, GSTT2
Zeta	GSTZ1
microsomal	MGST1, MGST2, MGST3

Subunit members can dimerize with members of the same class but not with members of other classes.

1.2.9.3. Properties and tissue expression of GSTs

Many of the early studies on GST developmental expression relied on substrate probes, and as such, conclusions drawn regarding differential expression suffer from a lack of specificity. Nevertheless, these studies did demonstrate differences in tissue-specific GST expression as a function of development.

Thus, using radioimmunological and immunohistochemical assays, were able to detect hepatic GSTA1 (182.4 to 247.2 pmol/mg cytosol protein) and GSTA2 (14.2 to 31.2 pmol/mg cytosol protein) expression as early as 10-weeks gestation. GSTA1 and GSTA2 expression levels increased 1.5- to 4-

fold, respectively, to adult levels within the first 1 to 2 years of life. GSTM also was detected but exhibited the lowest expression level in the fetal samples (1.3 to 2.4 pmol/mg microsomal protein) (Dufour and Svahn,2005).

1.2.9.4. GSTM1 glutathione S-transferase mu 1:

This gene encodes a glutathione S-transferase that belongs to the mu-class. The mu class of enzymes functions in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione. The genes encoding the mu class of enzymes are organized in a gene cluster on chromosome 1p13.3 and are known to be highly polymorphic. These genetic variations can change an individual's susceptibility to carcinogens and toxins as well as affect the toxicity and efficacy of certain drugs. Null mutations of this class mu gene have been linked with an increase in a number of cancers, likely due to an increased susceptibility to environmental toxins and carcinogens. Multiple protein isoforms are encoded by transcript variants of this gene(Dufour and Svahn,2005).

1.2.9.5. Glutathione S-transferase theta 1:

Glutathione S-transferase (GST) theta 1 (GSTT1) is a member of a superfamily of proteins that catalyze the conjugation of reduced glutathione

to a variety of electrophilic and hydrophobic compounds. Human GSTs can be divided into five main classes: alpha, mu, pi, theta, and zeta. The theta class includes GSTT1 and GSTT2. The GSTT1 and GSTT2 share 55% amino acid sequence identity and both of them were claimed to have an important role in human carcinogenesis. The GSTT1 gene is located approximately 50kb away from the GSTT2 gene. The GSTT1 and GSTT2 genes have a similar structure, being composed of five exons with identical exon/intron boundaries (Dufour and Svahn,2005).

1.2.9.6. Genetic polymorphisms:

Genetic polymorphism is the occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome resulting from mutation mechanisms in a gene or a chromosomal locus that create multiple forms (polymorphs) of that locus. Genetic polymorphism is a type of genetic variation in which individuals with sharply distinct qualities coexist as normal members of a population. The three main variation types at the DNA level are, single nucleotide changes (now named SNPs for single nucleotide polymorphisms), insertions or deletions (*Indels can be used as genetic markers in natural populations, especially in phylogenetic studies*) of various lengths ranging from one to several hundred base pairs and variations in the number of tandem repeats (Brattstrom, 1998).

Single base pair change (substitution! deletion! insertion of one nucleotide) at a specific locus, generally consisting of two alleles. Within a population, a minor allele incidence could be assigned for each (SNP) as the ratio of chromosomes in the population carrying the less common variant. In addition, in the past, single nucleotide polymorphisms with a minor allele frequency of (2: 1 %) were labelled "SNP". It is an essential to note that there are marked variants between human populations in term of distribution of SNP differences. For this cause, a SNP allele that is common in one geographical or ethnic group may be much less common. Several genetic polymorphisms have been documented for cytochrome P450 (CYP) and glutathione S-trans-ferase (GST) genes, representing either high or low metabolic activity or a deficiency of functional protein. The CYP and GST polymorphisms may affect the ability of enzymes to metabolize the chemical mutagens and carcinogens: As a result, it has been proposed that these differences in the ability to metabolize carcinogens may influence the susceptibility of individuals to leukaemia and cancer in general. Consequently, there may be an association between polymorphisms in genes encoding for xenobiotic-metabolizing enzymes and susceptibility to CML, (Brattstrom, 1998).

3-Drug metabolizing enzymes

The drug metabolizing enzymes system has been shown to influence the susceptibility, sequel and outcome of cancer treatment. These systems include the Glutathione transferases a family member of genes encoding enzymes involved in the metabolism of many chemicals and shown to be polymorphic with GSTM1 and GSTT1 being deleted in proportion of individuals where in the homozygous state results in a phenotypic absence of the corresponding enzyme. These enzymes are considerably important in the detoxification of many environmental compounds and reactive oxygen species, and hence may constitute important cancer predisposition genes. It includes also the cytochrome P450 enzymes one of the best studied for risk association with cancer (Aqundez, 2004). The P450 shows conflicting and variable degree of association with cancer, possibly reflecting, variation in the role played by these enzymes in carcinogenesis and the genetic background of the population.

In some populations in the Indian subcontinent, the frequencies of homozygous 3/3 genotype and CYP3A5*3 allele were elevated significantly in the CML group compared to controls ($\chi^2=93.15$, $df=2$, $P=0.0001$) (Sailaja *et al.*, 2010).

Increased risk of AML has also been reported for combined polymorphisms in detoxification and DNA repair enzymes. Voso *et al.*, (2007), and patients that achieved complete molecular response following

administration of imatinib showed significantly ($p=0.013$) higher in vivo CYP3A activity than patients achieving partial molecular response (Green *et al.*, 2010).

1.3. Rationale

This study is hypothesized that patients who have CML and AML genotypes that encode for high-activity enzymes would have shat survival and decreased incidence of adverse effects in comparison with patients with genetically determined, low or non functioning detoxification activity. Futherly, the study will measure the association between Gulutathione S-Transferases (GST) T1 and (GST)M1, polymorphisms and the clinical outcomes in CML and AML patients.

Genetic screening of the patients with CML and AML may provide new diagnostic methods for early deletion of the disease.

No published data regarding this problem was available in Sudan so the result of this study can be obtained data yield generally screening and abnormal of GST enzyme in CML and AML patients.

1.4. Objectives

General objective:

Molecular detection of Polymorphisms of Glutathione S-Transferases (GST) M1 and T1 Genes in Sudanese Patients with Myeloid Leukaemia in Khartoum State

Specific objective:

1. To detect the frequency of GST (T1) and (GST) (M1) in myeloid Leukaemia.
2. To investigate the role of gene environment interaction in terms of (GSTT1) and (GSTM1) genes (alone or in combination) in modulation of (myeloid leukemia) (CML and AML).
3. To determine the role of gene deletion of xenobiotic-metabolizing enzyme (GSTT1 and GSTMI) in the development of (myeloid leukemia) (CML and AML).

CHAPTER II

2. Materials and Methods

2.1. Study Design

The design of this study is a 1) descriptive 2) cross-sectional, 3) hospital-based study 4) prospective study.

2.2. Study population

All patients according to nationality they will be different ethnic group with CML and AML attended Radiation Isotope Centre Khartoum Hospital (RICK) were enrolled in this study.

30 Patients diagnosed with chronic myeloid leukemia.

30 Patients diagnosed of acute myeloid leukemia.

30 Controls with matched (health individual).

Material and Methods:

Ethical sample size:

I am take orally to the patients and collected and acceptable to collect this sample.

2.4. Sampling: three ml of whole blood was transferred in E.D.T.A collected.

2.5. Data Collection

Was designed to collect demographic data (Appendix 2) shows the

questionnaire by each patient. The patient data records, sample collection: logistic and patient safety issues, were handled according to the protocols set out by University of Sudan.

2.6. Samples collection:

Sudanese patients with CML and AML who were diagnosed using full blood count, Bone marrow examination and genotyping. These will be taken with right ethical approval.

The patient specimens will be collected from the diagnosed patients with CML and AML. Each sample was processed as follows: Venous blood and BM were used to diagnose all patients and the PCR test was used for CML and AML patients as a confirmatory test.

(1) Complete haemogram complete blood count

Stain: Wright stain.

PB: Peripheral blood

BM: Bone marrow slides

Method:

1. Fixation by 2ml wrights stain in steady rack, for 4 minutes.
2. Was added 2 ml buffer (D.W.) let for 10 mints.
3. was dry and comment in vitro scope.

Reagents:

1. Wrights stain.
2. Distilled water.

Principles:

Staining of granules differentially depends on two components; a zure B and eosin and methylene blue which enhance the staining of nucleoli and polychromatic red cells.

Cytochemical stain:

The principal uses of Cytochemistry are.

- 1- To characterized the blast cells in acute leuskaemias as myeloid.
- 2- To identify granulocytic and monocyclic components of acute myeloid leukaemias.
- 3- To indentified unusual lineages occasionally involved in colonel's myeloid disorders e.g. basophiles and must cell.
- 4- To detected of cytoplasmic abnormalities and enzyme deficiencies in myeloid disorders e.g. neutrophil alkaline phosphatase deficient neturophils in chronic myeloid leukemia (CML):

- 1- Sudan Black (B).
- 2- Neutrophil alkaline phosphates (NAP).
- 3- Toluidine Blue Stain.
- 4- Myeloperoxidase (MPO).

Sudan Black (B):

Sudan black (B) is a lipophilic dye that binds irreversibly to an undefined granule component in granulocytes, eosinophils and some monocytes. It cannot be extracted from the stained granules by organic dye solvents, and

gives comparable information to that of MPO staining the currently used staining solution is essentially than described by Sheehan of storey in (1974).

Prouder:

Reagents:

Fixative 40% formaldehyde solution stain. Sudan black (B) 0.3gm in 100ml absolute ethanol phenol buffer. Was dissolved 16g crystalline phenol in 30ml absolute ethanol adds to 100ml distilled water in which 0.3g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}_2$ has been dissolved. Working stain solution. Add 40ml butter to 60ml Sudan black (B) solution.

Method:

- 1- was fixed air-dried smears n formalin vapour as follows: place a small square of filter paper in the bottom of a coupling jar. Add 2 drops of 40% formalin, put on the lid and leave for 15mn to allow vaporization – place the slides in the coupling jar and replace the lid. After 5-10 mints, was removed the slides and stand on end for 15 mints to air-wash.
- 2- Was immersed the slides in the working stain solution for 1h in a coplin-jar with a lid on.
- 3- Was a Transferred slide to staining rack and immediately flood with 70% alcohol. After 30s, tip the 70% alcohol off and flood again for 30s. Repeat three times in total.
- 4- Was rinsed in gently running tag water and air-dry.

5- Was Counterstained without for the fixation with Leishman stain or may-Grunwald-Giemsa.

Neutrophil Alkaline Phosphates (NAP):

Alkaline phosphates activity is found predominantly in mature Neutrophil, with some activity in metamyelocytes. The most significant diagnostic use of the NAP score is in chronic myeloid leukemia (CML and AML). In the chronic phase of the disease the score is almost invariably low, usually zero.

Method:

Reagent: Fixative 4% formalin methanol. Add 10ml 40% formalin to 90ml methanol keep at -20°C or in the freezer compartment of a refrigerator. Discard after 2 weeks substrate – Naphthol as phosphate store in freezer. Buffer 0.2mol/c Tris buffer PH 9 stock substrate solution. Dissolve 30mg naphthol as phosphate in 0.5ml N,N dimethyl-formamide. Add 100ml 0.2 mol/l tris buffer, PH 9.1. Store in refrigerator at $2-4^{\circ}\text{C}$ the solution is stable for several months.

Coupling a 20-dye. Fast Blue BB salt. Store in freezer.

Counter stain neutral red, 0.02% aqueous solution.

Method:

1- Was Fixed freshly made air-dried blood films for 30s in cold 4% formalin in methanol.

2- Was rinsed with tap water and air-dry.

- 3- Was prepared working substrate solution by allowing 40ml of stock substrate solution to warm to room temperature. Add 24mg of fast Blue BB and mix thoroughly until dissolved. Incubate slides for 15min.
- 4- Was washed in tap water and air-dry.
- 5- Was Counterstained for 3 min in 0.02% aqueous neutral red, rinse briefly.

Method:

Reagent: Toluidine blue 1% W/V in methanol. Add 1g of toluidine blue to 100ml methanol and mix for 24h on a roller or with a magnetic flea the stain is stable indefinitely at room temperature. Keep tightly stoppered.

Method:

- 1- Was placed air-dried smears on a staining rack and flood with toluidine blue solution.
- 2- Was incubated for 5-10mins. Rinse briefly in gent

Molecular Methods:

1. DNA extraction(salting out method)
2. PCR
3. Electrophoresis

DNA extraction from human blood (Salting out method):

II. Solutions and buffers:

- PBS (1 mM KH₂PO₄, 154mM NaCl, 5.6 mM Na₂HPO₄; pH7.4)- 1 liter:

- Sucrose Triton X-lysing Buffer

1 M Tris-HCl, pH8

1 M MgCl₂

Triton X-100

Made up to 100ml was distilled water, Autoclaved and kept at 4 degrees

11 g sucrose (D (+) saccharose) was added just before use (11 g/100mL).

The solution cannot be stored with sucrose

T20E5, pH8

20 mM Tris-HC

5 mM EDTA

Proteinase K (stock of 10 mg/mL)

Saturated NaCl

100 mL of sterile water was taken and 40g NaCl was added to it slowly until absolutely saturated. It was agitated before use and NaCl was let to precipitate out.

Procedure:

Purification of DNA from frozen blood (for 2. 5 ml sample). The blood was thawed at room temperature 37 °C and then transferred to a sterile polypropylene tubes then it was diluted with 2 volume of PBS (1 mM KH₂PO₄, 154 mM NaCl, 5.6 mM Na₂HPO₄, pH 7.4) mixed by inverting the tubes and centrifuged at 2200 g for 10 minutes. The supernatant was poured off and the pellet (reddish) was resuspended in 9 volume 12,5ml of

Sucrose Triton X-100 Lysing Buffer (1 M Tris-HCl 10 ml, 1 M MgCl₂ 5 ml Triton X-100 10 ml Made up to 1 liter with distilled water). Sucrose was added just before use i.e. (11 g/100 mL). The mixture was vortexed and then placed on ice for 5 minutes. The mixture was spun for 5 minutes at 2200 g (2300 rpm in TH.4 rotor) and the supernatant was poured off. The pellet pinkish was resuspended in 1.5 mL of T20E5 (0.6X volume of original blood). 10% SDS was added to a final concentration of 1% (100 µL) then Proteinase K was added (10 mg/mL) to a final concentration of 250 µg/mL (35 µL). The mixture was mixed by inversion after adding each solution then the samples were incubated at 45°C overnight. 1 ml of saturated NaCl was added to each sample and mixed vigorously for 15 seconds then it was spun for 30 minutes at 2400 g. A white pellet was formed which consisted of protein precipitated by salt, the supernatant that contained the DNA was transferred to a new tube and precipitation of DNA was achieved by adding two volumes of absolute alcohol kept at room temperature. The solution was agitated gently and the DNA was spooled off and transferred to eppendorf tube. The DNA was washed in 70% ice-cold alcohol (1 mL), air dried and was dissolved in the appropriate volume of TE (100-250 µL) and stored at 4 degrees overnight to dissolve.

2.6.2. Polymerase chain reaction (PCR):

The experiment consist of experimental DNA and negative control in 0.5 ml PCR tube all of the components necessary to make new strands of DNA in the PCR process will be placed in 20ul total volume Primers sequences:

Table (2.1.): Gene name, primer sequence, annealing temperature and PCR product length (Taspinar and Aydos,2008)

Gene	Primer sequence	AT (°C)	Leng. PCR (bp)
GSTT1	Fwd 5'-TTC CTT ACT GGT CCT CAC ATC TC-3'	60	459
	Rev 5'-TCA CCG GAT CAT GGC CAG CA- 3'		
GSTM1	Fwd 5'-GAA CTC CCC TGA AAA GCT AAA GC-3'	60	215
	Rev 5'-GTT GGG CTC AAA TAT ACG GTG G- 3'		
HAS	Fwd 5'GCC CTC TGC TAA CAA GTC CTA-3'	60	35
	Rev 5'-GCC CTA AAA AGA AAA TCC CCA ATC- 3'		

Polymerase chain reaction procedure for GSTT1 and GSTM1

amplification gene:

- **Initialization step:** This step consists of heating the reaction to a temperature of 94 °C which is held for 5 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.

- **Denaturation step:** This step is the first regular cycling event and consists of heating the reaction to 94°C for 1 minute. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
- **Annealing step:** The reaction temperature was lowered to 60 °C for 1 minute allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the T_m of the primers used. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.
- **Extension/elongation step:** The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 72 °C .At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to

be amplified. 36 cycles required for GSTT1 & GSTM1 amplification (annealing, denaturatin and extension)

- **Final elongation:** This single step was occasionally performed at a temperature of 72 °C for 5minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Table (2.2): The PCR program used for the GSTM1 and GSTT1 genes (Taspinar and Aydos,2008).

Gene	First denaturation (°C)	Second denaturation (°C)	Annealing (°C)	Extension (°C)	Final extension (°C)	Cycles
GSTT1	94	94	55	72	72	35
GSTM1	94	94	55	72	72	35

Agarose gel electrophoresis:

Agarose gel electrophoresis is the easiest and commonest way of separating and analyzing DNA. The purpose of the gel might be to look at the DNA, to quantify it or to isolate a particular band. The DNA was visualized in the gel by addition of ethidium bromide. This binds strongly to DNA by intercalating between the bases and is fluorescent meaning that it absorbs invisible UV light and transmits the energy as visible orange light.

Method:

Making the gel (for a 1.5% gel, 50mL volume)

1. was weighed out 0.8g of agarose into a 250mL conical flask. Add 50mL of 1xTBE , swirl to mix.
2. Microwave for about 1 minute to dissolved the agarose.
3. Was leaved it to cool on the bench for 5 minutes down to about 60°C (just too hot to keep holding in bare hands).
4. was added 1-4 µL of ethidium bromide (10mg/mL) and swirl to mix (*. Ethidium Bromide is **mutagenic** and should be handled with extreme caution*)
5. was poured the gel slowly into the tank. Push any bubbles away to the side using a disposable tip. Insert the comb and double check that it is correctly positioned.
6. was leave to set for at least 30 minutes, preferably 1 hour, with the lid on if possible.
7. was pour 1x TBE buffer into the gel tank to submerge the gel to 2–5mm depth. This is the running buffer.

Loading of sample

1. For PCR reactions, it depends on the PCR but in routine applications 10–20µL should be plenty to see the product on the gel.

2. Load the first well with marker.
3. Continue loading the samples and finish off with a final lane of marker
4. was closed the gel tank, switch on the power-source and run the gel at 5V/cm.
5. was checked that a current is flowing
6. was monitored the progress of the gel by reference to the marker dye.
7. was switched off and unplug the gel tank and carry the gel (in its holder if possible) to the dark-room to look at on the UV light-box.

CHAPTER III

Results

This study was collected in RICK to assess the role of the gene deletion of the detoxification enzymes glutathione S- transferencees GSTMI and GSTTI in the development of myeloid leukemia in Sudan. The study included 90 subjects (30 patients with acute myeloid leukemia, 30 with chronic leukemia and 30 healthy controls).

According to this study most of the AML and CML patients were from Khartoum State (where RICK) is located followed by the western region in case of CML. Most of the AML patients were between the age of (40-50) years on the other hand the age of (40-50) years on the other hand most of the CML patients the controls were between (31 – 40) years old. The study revealed than both AML and CML patients are mostly from the Afro-asiatic population group, followed by Nile-Saharan in case of AML. In this study the male / female ratio of AML was (1:2) while that of CML was (2:1), the control group included males and females (male / female ratio is equal. Genotyping of patients showed that only 2 of the AML patients, 7 of the CML patients and 8 controls were detected for the GSTMI while 22 of the AML patients 23 of the CML patients and 11 controls were detected for the GSTTI. For deletion in GSTMI, AML 6.67% of GSTMI, 23% of CML and 26.67% of controls were deleted on

the other hand for GSTT1 and only 73% of AML 76.67% of CML and only 36.67% of controls were deleted.

Coloration:

They are relationship between deletion of Gens GSTT1, GSTM1 and myeloid leukemia. The presents of myeloid leukemia the deletion of Gens GSTT1, GSTM1 and absent of myeloid leukemia the presents of Gens GSTT1, GSTM1 in this studies, P. value of GSTT1 is high significant (0.001), in CML and AML.

Method of data analysis:

SPSS version 19.0 used to obtained SPSS analyze the data, this is a computer program called Statistical Product and service solution. The results presented in form of frequency and percentages.

Table No (3.1) Disabler of study population to the age of Myeloid leukemia

	Age Years								Total
	1-10 y	11-20 y	21-30 y	31-40 y	41-50 y	51-60 y	61-70 y	71+ y	
A.M.L	1	1	6	5	8	5	2	2	30
C.M.L	1	3	6	7	9	1	3	0	30
Control	5	3	8	10	2	1	0	1	30
Total	7	7	20	22	19	7	5	3	90
P. Value = .045									

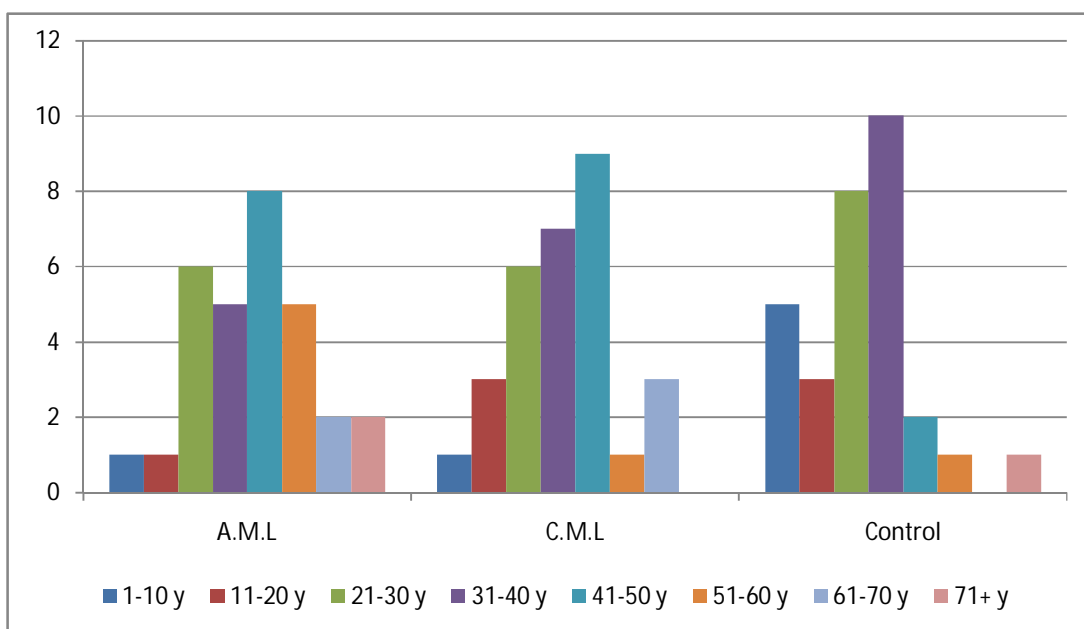


Figure 3.1: Frequency of study population according to the age

Table No (3.2) Disabler of study population to the residence

Count		RESIDENT					Total
		Westrn Region	KH	Central Region	Northern Region	Estern Region	
DIAGONSI	A.M.L	3	18	5	2	2	30
	C.M.L	9	10	6	5	0	30
	CONTROL	1	24	3	0	2	30
Total		13	52	14	7	4	90

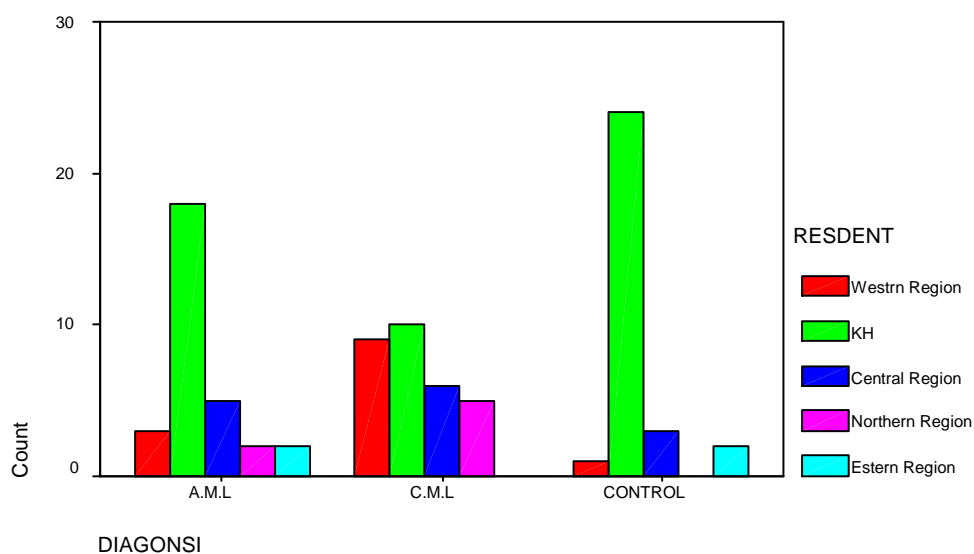


Fig (3.2) Frequency of study population according to the residence

Table No (3.3): Compare between study populations according to the tribe of Myeloid leukemia CML, AML and control cases:

Count		Tribe			Total
		Afro- asiatic	Nile- Saharan	North- Kordofan	
DIAGONSI	A.M.L	3	12	15	30
	C.M.L	7	7	16	30
	CONTROL	14	6	10	30
Total		24	25	41	90

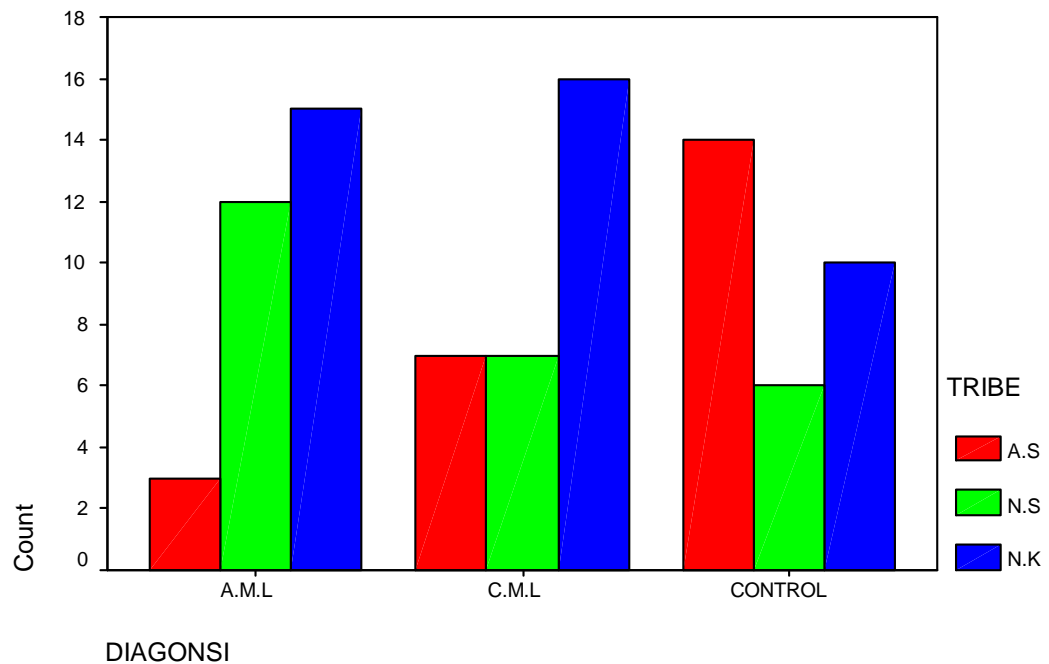


Fig (3.3) compare between study populations according to the tribe of Myeloid leukemia

Table No (3.4) Diagnosis and gender crosstabulation CML, AML and control cases:

DIAGONSI * GEDER Crosstabulation

Count		GEDER		Total
		f	m	
DIAGONSI	A.M.L	19	11	30
	C.M.L	10	20	30
	CONTROL	17	13	30
Total		46	44	90

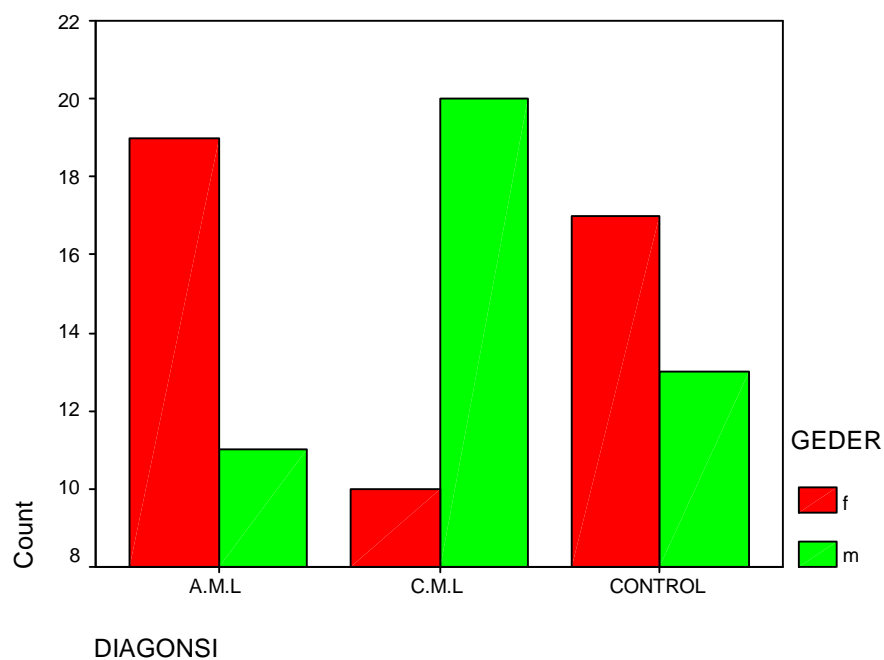


Fig (3.4) Diagnosis and gender crosstabulation

Table (3.5) Compression between Groups (case and control) GSTM1 gene Crosstab

		GSTM1		Total
		+ve	-ve	
GRUOP	Case	46	14	60
	Control	27	3	30
Total		73	17	90

P –value =0.128

Table (3.6) Comparison between Groups (case and control) GSTT1 gene Crosstab

		GSTT1		Total
		+ve	-ve	
GRUOP	Case	18	42	60
	Control	20	10	30
Total		38	52	90

P –value =0.001*

CHAPTER IV

4.1 Discussion

This study was done in RICK during the period 2011 to 2012 to assess the role of the gene deletion of the detoxification enzymes glutathione S-transferases GSTMI and GSTTI in the occurrence in Sudan. The study included 30 patients with acute myeloid leukemia, 30 patients with chronic leukemia and 30 healthy controls.

According to this study most of the AML and CML patients were from Khartoum State (where RICK) is located followed by the western region in case of CML.

The AML patients were between the age of (40-50) years on the other hand most of the CML patients were between (40-50) years the controls were between (31 – 40) years old.

The study revealed that both AML and CML patients were from the Afro-asiatic population group, followed by Nile-Saharan in case of AML.

In this study the male / female ratio of AML was (1:2) (36.7-63.3%) while that of CML was (2:1) (66.7%-33.3%), the control group included males and females (male / female ratio is equal (43.3%-56.7%) equal.

Genotyping of patients showed that only 2 of the AML patients, 7 of the CML patients and 8 controls were detected for the GSTMI while 22 of the AML patients 23 of the CML patients and 11 controls were deleted for the GSTTI.

For deletion in GSTMI, AML 6.67% of GSTMI, 23% of CML and 26.67% of controls were deleted on the other hand for GSTTI and only 73% of AML 76.67% of CML and only 36.67% of controls were deleted GST T1 gene is high significant P. value for CML and AML, that means GSTT1 gene is risk factor for Myeloid leukemia.

4.2. Conclusion

Polymorphism of carcinogen-metabolizing enzymes, GSTT1 is a RISK factor for Myeloid leukemia in among Sudanese because the P. value of the Glutathione S-Transferases GST T1 gene is high significant of P. value and GSTT1 is a risk factor for Leukemia among Sudanese.(P. value = 0.001).

1. GSTT1 gene deletion was obtained in CML (76.67%) was deleted and AML (37%) OF GSTT1 was deleted.
2. The ration of male: female in CML (2:1) while male: female(1:2).
3. AML and CML is common type in Sudan.

Review studies.

In Sudan impact of the distribution of the GSTM1 and GSTT1 genotype leukemic patients was studied in 77 leukemic patients and 107 controls by Tagelsir et al., (Submitted). The results suggest that these genotypes could play a role in the development of leukemia particularly AML. Statistical analysis showed a significant preponderance of null genotype of both genes among pooled cases [GSTM1 OR 3.45 (95% CI, 1.65 - 7.19); P = 0.001] for; GSTT1 OR 8.57 (95% CI, 3.68 -19.93); P < 0.0001]. Double null was also higher in patients compared to controls (P = 0.01). When the cases were stratified according to the disease type, AML showed the highest positive predictive value for both loci (GSTM1 P < 0.0001, GSTT1 P < 0.0001), CML displayed the least positive

predictive value for GSTM1 (0.02), while for GSTT1 the result was as same as AML ($P < 0.0001$).

The Tagelsir study suggests an increased risk for leukemia associated with GSTM1 and GSTT1 null genotypes and highlights a potential role of genetic makeup in leukaemogenesis. The most statistically significant association for GSTT1 observed for both AML and CML may highlight a possible role of GSTT1 enzyme in protection of the myeloid series. Allelic variation in the gene encoding the GST isoform theta (GSTT1) enzymes was found to modulate the rate of benzene metabolism and excretion Rossi et al., (1999) as well as benzene-induced myelotoxicity Wan et al., (2002) Chen et al., (2007). AML – which showed the strongest association with both genes– comprises a distinct type of leukemia with different subtypes and is shown to be to somewhat associated with environmental exposure.

4.3. Recommendations

- Analysis of a larger number of individuals is necessary to further confirm these results which may provide a useful public health approach for early detection and prevention of leukemia.
- Additional studies should be undertaken to fully determine GSTM1 and GSTT1 contribution to the pathogenesis of leukemia.

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APPENDIX 1.

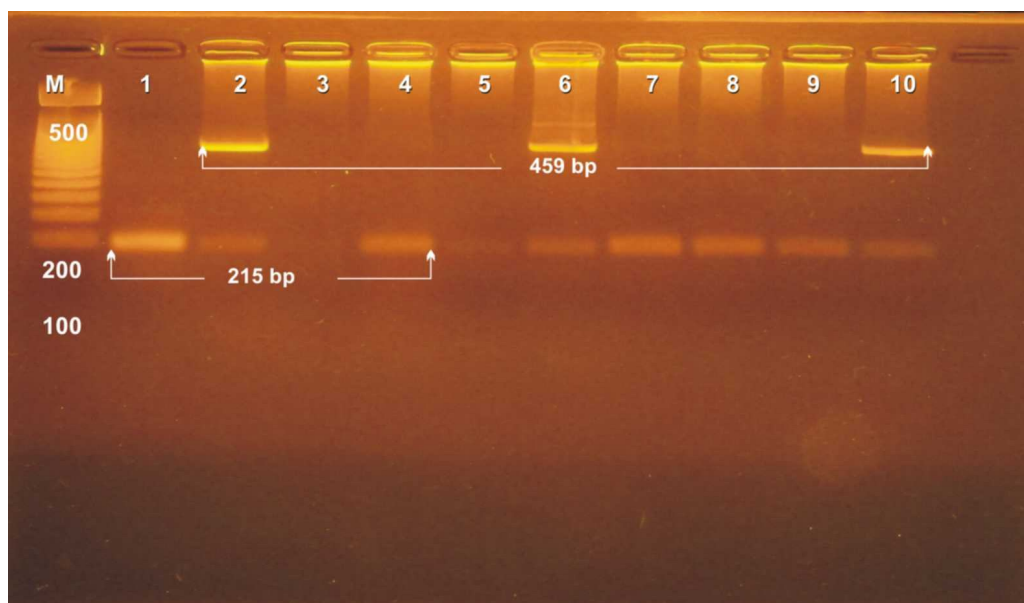


Fig 1: M: DNA Marker 2,6,10 shown cutband GSTT1 459 bp, AND SHOWING 1 ,4,7,8,9, SHOWING CUTBAND GSTM1 215 bp

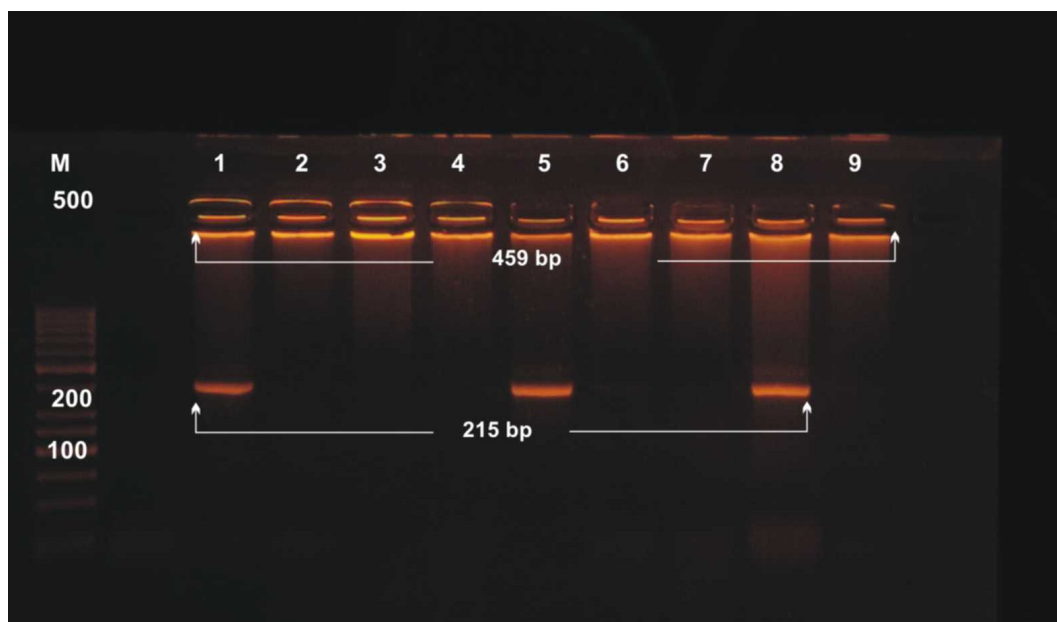


Fig 2: M: DNA Marker Lane 1,5,8 positive Gane GSTM1,
DNA marker lane 2,3,4,6,7,9 positive Gane GSTT1