

CHAPTER ONE

Introduction

1.1 General Introduction:

The acute leukaemias are characterized by proliferation of immature cells, either lymphoid or myeloid, with a failure of differentiation to mature end cells. Because the immature cells are proliferating in the bone marrow they replace normal haemopoietic cell (Bain, 2004).

Acute myeloid leukemia (AML) results from the malignant transformation of a bone marrow (myeloid) progenitor cell or stem cell, which is the normal precursor for granulocytes, erythrocytes, and megakaryocytes. The traditional classification of the acute leukemias has relied on morphologic description, reflecting the predominant cell type present within the bone marrow population and relating that cell to its normal hematopoietic counterpart. This system was based solely on light-microscopic evaluation of routinely stained blood and marrow smears, supplemented by a limited number of cytochemical procedures. In 2001, a committee of the World Health Organization described a comprehensive classification scheme that utilizes morphology, immunophenotyping, etiology, and cytogenetics and more clearly distinguishes between AML and other myeloproliferative disorders. A diagnosis of AML is established when 20% or more of the nucleated marrow cells are blast cells. Clonal chromosomal abnormalities can be detected in most cases of AML. Particular abnormalities correlate with specific morphologic subtypes and clinical profiles. These cytogenetic abnormalities are somatic (rather than germ line) mutations that frequently result from translocations of chromosomal DNA, resulting in new (abnormal) protein products from the resultant fusion genes. It is assumed that the protein products from these fusion genes are responsible for the cellular dysregulation that leads to the malignant state. Such recurring chromosomal abnormalities are critical in determining therapeutic strategy and have provided important independent information regarding response to therapy and overall prognosis. Genes known to affect the outcome in patients with AML include *FLT3*, *KIT*, *CEBPA*, *BAALC*, *ERG*, *MLL*, and *NPM1* (Crowther *et al*, 2008).

It is strongly recommended that cytogenetic analysis be performed before initiation of therapy on every newly diagnosed patient because studies of the prognostic significance of recurring cytogenetic abnormalities in AML have yielded consistently similar results. Thus, in many centers, plans for postremission therapy rely heavily on cytogenetic analysis at diagnosis. Cytogenetic data have been used to map chromosomal breakpoints at a molecular level, allowing for the use of more sensitive techniques, including probes for fluorescence in situ hybridization and primers for reverse transcriptase polymerase chain reaction. However, both of these methods test only for specific, defined genetic mutations and are not used initially for general screening or for a comprehensive evaluation (Crowther *et al* ,2008).

Acute myeloid leukaemia occurs at all ages from the neonatal period to old age. However, the incidence increases steadily (Drew, 2003).

1.2 Rationale:

Myeloid leukemia's is fatal diseases and have increasing prevalence in sudan (Tambal *et al* ,1996).There is many studies revealed association of acute myeloid leukemia's with certain abnormality. Inherited of alleles (KIT D816) result in increased proliferation of cells due to uncontrollable activation of tyrosine kinase receptors that lead to poor prognosis of patients under chemotherapy treatment. To know that there are published reports about the association between KIT D816 mutation and acute myeloid leukemia's outcome but no published paper in Sudan about this article so our study will be done to detect this polymorphism and its association with myeloid leukemia's outcome among Sudanese patients in Khartoum state.

Therefore in this study will detect KIT D816 by ACB-PCR technique and then follow up these patients under chemotherapy treatment by use CBC test and clinical features of them within 4 months as minimum period from developed AML to evaluate the outcome.

1.3 Objective:

1.3.1 General objective:

To study KIT D816 mutation and its association with outcome in Sudanese acute myeloid leukaemia patients at Khartoum state .

1.3.2 Specific objectives:

- To detect frequency of KIT D816 mutation among Sudanese patients with AML by use Allel Specific Competitive Blocker PCR Assay .
- To evaluate the impact of KIT D 816 mutation on outcome of AML .
- To determine association between KITD816 mutation with patient's age and gender.
- To determine effect of KIT D816 mutation with different types of AML in outcome of patients under treatment.

CHAPTER TWO

Literature review

2.1 Acute Myeloid leukemia :

AML is malignant, clonal disease that involves proliferation of blasts in bone marrow, blood, or other tissue. The blasts most often show myeloid or monocytic differentiation. Almost 80% of patients with AML will demonstrate chromosome abnormalities, usually a mutation resulting from a chromosomal translocation (the transfer of one portion of the chromosome to another). The translocation causes abnormal **oncogene** or tumor suppression gene expression, and this results in unregulated cellular proliferation. Genetic syndromes and toxic exposure contribute to the pathogenesis in some patients. Although the diseases grouped into the acute myelogenous leukemia categories have similar clinical manifestations, the morphology, immunophenotyping, and cytogenetic features are distinct. Cytochemical stains are used along with morphology to help identify the lineage of the blast population. Electron microscopy may also be used to subclassify the various leukemias. When morphology and/or **cytochemistry** evidence of lineage is absent, flow cytometry is used to specifically tag the myeloid or lymphoid antigens and thus classify the acute leukemias (Cisela ,2007).

2.1.1 Epidemiology:

Although acute leukemias are infrequent diseases, they are highly malignant neoplasms responsible for a large number of cancer-related deaths. Acute myeloid leukemia (AML) is the most common type of leukemia in adults, yet continues to have the lowest survival rate of all leukemias. While results of treatment have improved steadily in younger adults over the past 20 years, there have been limited changes in survival among individuals of age >60 years ((Estey *etal*,2008).

2.1.2 Incidence of AML:

2.1.2.1 International incidence of AML:

It is estimated that 44240 individuals in the USA will be diagnosed with one form of leukemia. Approximately 21790 will die of their disease

Although the incidence of acute leukemias accounts for less than 3% of all cancers, these diseases constitute the leading cause of death due to cancer in children and persons younger than 39 years of age .AML accounts for approximately 25% of all leukemias in adults in the West and constitutes the most frequent form of leukemia . Worldwide, the incidence of AML is highest in the USA, Australia, and Western Europe. The age-adjusted incidence rate of AML in the USA is approximately 3.7 per 100000 persons (= 2.6 per 100000 when age-adjusted to the world standard population). In the USA, 13410 men and women (7060 men and 6350 women) are estimated to be newly diagnosed with AML in 2007 (Estey *et al*,2008).

The incidence of AML increases with age, accounting for 80% of acute leukemias in adults and for 15% to 20% of acute leukemias in children. Of note, however, is that when congenital leukemia (occurring during the neonatal period) does rarely occur, it is paradoxically AML rather than ALL and is often monocytic. The rate of AML is somewhat higher in males than females, and there is an increased incidence in developed, more industrialized countries. Eastern European Jews have an increased risk of developing AML, whereas Oriental populations have a decreased risk. The high incidence of individuals having congenital defects such as Down syndrome and bone marrow failure syndromes such as Fanconi's anemia has demonstrated that these factors are often implicated in the pathogenesis of AML. It has also been well documented that leukemia is associated with exposure to ionizing radiation, as this was most notably reported with the increase in leukemia that occurred following the release of atomic bombs over Hiroshima and Nagasaki in 1945. The fallout from atomic bombs and exposure to nuclear reactor plants has caused much well-founded public apprehension, fear, and concern over the past 50 years. A wide variety of chemicals and drugs have been linked to AML. In a study involving factories in China, the risk of developing leukemia was five to six times higher in workers with recurrent exposure to benzene than in the general population. Many drugs, in particular, therapy-related alkylating drugs, are associated with AML emerging after the treatment. All of the chronic myeloproliferative disorders (chronic myelocytic leukemia [CML], idiopathic myelofibrosis [IMF], polycythemia vera [PV], essential thrombocythemia [ET]) have an

increased propensity for terminating in AML, with 60% to 70% of CML cases undergoing a transition to AML (Cisela, 2007).

2.1.2.2 Incidence in Sudan:

In 184 cases of leukaemia .CML together with ALL were the most frequent types comprising 125 of the cases (67.9%).65 cases (35.3%)of CML were found among Sudanese .60% cases of ALL were encountered (32.6%). It showed an equal distribution in males and females of 1:1 ratio. The peak of incidence was found to be in the first decade of age .the incidence decreased at the seventh decade of age. It was found to be more in men with a male to female ratio of 1.2:1 .Where 21 cases were reported with a sharp decrease in incidence in the third decade, 36 cases of CLL (19.6%) were recorded, with a marked difference in incidence between male and female. The study showed ALL ranked the second in frequency among all leukaemia (32.6%) constituting 72.3% of all acute leukaemia among Sudanese. The same finding was reported in the same Sudan (23.4%).A high frequency of ALL was reported from Uganda (33%) and Jordan 45.7%in sharp contrast to what was reported from Kenya where ALL formed only 15%among leukaemia .In this series the male to female ratio was 1.2:1 ratio in both Jordan and USA (Tambal *etal*,1996).

All has a bimodal peak of incidence early peak in the first decade and late peak from middle age on wards .this study showed the first peak in the decade of age but the second peak was non-existent (Tambal *etal*,1996).

CLL was found to be the third type in frequency 36 patients were found forming 19.6%of all leukaemia in Sudanese .CLL came the second in frequency in a study carried out the Sudan (36.2%). and the fourth in frequency in another one (13.3%).An occurrence close to that reported from Jordan (9.3%) and Kenya (19.3%) which was in sharp contrast to its occurrence in Western Countries where it was reported to be the commonest type of all leukaemia. Concerning the age distribution, no patient with CLL was found below the age of 20. The incidence showed rise at the 5th decade with a maximum of incidence at the 7th decade of age .Most of the patients (75%) above 50 years of age. In Jordan and western countries the percentage of patients above 50 years of age was 67% and 90% respectively. In our materials men were affected more than

women with 4.1:1 male to female .Previous studies in the Sudan reported 3.9:1 and 3:1 male to female ratio .Ratio that was reported to be 2:1 was in base Jordan and western countries (Tambal *etal*, 1996).

AML was the least frequent type of leukaemia (12.5%) in this series forming only 27.7% of the acute leukaemia in Sudanese .but it was the third in frequency (17.2%)and (10.6%).in earlier studies carried out in the second frequent type (31.5%)among all leukaemia in Jordan . Most of the patients with AML in this study (60.9%) were below 40 years of age .It showed that it is a disease of young Sudanese with a peak of incidence in the third decade similar to that reported from Jordan. This finding was in clear contrast to that reported from the USA where the incidence of AML was raised after the age of 50 years with a peak between 75 and 85 years of age. In this study there was a female predominance with a 1:19 male to female ratio. Contrary to that was reported from the USA and Jordan where males showed predominance (Tambal *etal*,1996).

In conclusion CML and ALL were the most frequent seen and AML was the least frequent type of leukaemia in this study .AML seemed to affect younger age groups. No aetiological study accompanied this series or investigation. However variation in frequency of leukaemia type and age associated distribution observed in our study and the variations noticed from other countries suggest that environmental influences may play an important role in the causation of leukaemia (Tambal *etal*, 1996).

In 2009, the first National Population-based Cancer Registry (NCR) was established in Sudan. The first data from the NCR for Khartoum State for the period 2009-2010 conducted by (Intisar *et al*, 2014).

The NCR staff used passive and active approaches to collect data on cancer diagnosed by all means in Khartoum State. Rates were age standardized to the 2010 Sudan Standard Population and 1966 and 2000 World Standard Population and expressed per 100,000 populations. During 2009-2010, 6771 new cancer cases were registered. Of those, 3646 (53.8%) cases were in women and 3125 (46.2%) were in men. The most commonly diagnosed cancer among women was breast followed by leukemia, cervix, and ovary, and among men it was prostate cancer followed by leukemia, lymphoma, oral, colorectal, and liver. In children less than 15 years of age, leukemia was the most common cancer followed lymphoma, and cancer of the eye, bone, kidney, and the brain.

The overall age-standardized rate (ASR) per 100,000 population was higher in women (124.3) than in men (90.8) using 2010 Sudan Standard Population. Similarly, it was higher in women (188.6 and 206.3 per 100,000 population) than in men (145.4 and 160.0 per 100,000 population) using 1966 and 2000 World Standard Population, respectively. The data from NCR indicated that prostate and breast as the most commonly diagnosed cancer sites in men and women in Khartoum, while cancer of the cervix trailed behind portraying a cancer picture similar to that of the developed world. Despite the study limitations, the NCR data gave a fair representation of cancer profile of Khartoum State and underscored the need for high-quality cancer registries in Sudan (Saeed *et al*, 2014).

2.1.3 Etiology:

The development of AML has been associated with several risk factors. Remarkably though, as of yet defined risk factors account for only a small number of observed cases. These include age, antecedent hematological disease, genetic disorders as well as exposures to viruses; radiation, chemical or other occupational hazards, and previous chemotherapy. The development of leukemia is a process consisting of multiple single steps that requires the susceptibility of a hematopoietic progenitor cell to inductive agents at multiple stages. The different subtypes of AML may have distinct mechanisms, suggesting a functional link between a particular molecular abnormality or mutation and the causal agent. In most cases of AML the malignancy arises *de novo* and no leukemogenic exposure can be deciphered (Estey *et al*, 2008).

2.1.3.1 Genetic Factors:

Genetic disorders and constitutional genetic defects are important risk factors associated with AML in children. Children with Down syndrome have a 10- to 20-fold increased likelihood of developing acute leukemia. Other inherited diseases associated with AML include Klinefelter's syndrome, Li-Fraumeni syndrome, Fanconi anemia, and neurofibromatosis. Furthermore, risk factors for developing AML in children were identified and include race/ethnicity, the father's age at time of conception, and time since the mother's last live birth. Islander children had a higher risk than non-Hispanic white infants; children born to fathers older than 35,

compared to those aged 20–34, had an increased risk; and longer time since the last live birth (at least 7 years) resulted in an increase risk (Estey *etal*,2008)

2.1.3.2 Acquired Genetic Abnormalities:

Acquired (“somatic”) clonal chromosomal abnormalities are found in 50–80% of AML with rising incidences in patients with secondary leukemia or older age. Frequently found abnormalities include loss or deletion of chromosome 5, 7, Y, and 9, translocations such as t(8;21)(q22;q22); t(15;17),(q22;q11), trisomy 8 and 21, and other abnormalities involving chromosomes 16, 9, and 11. Cytogenetic abnormalities constitute at present the most important predictors of short- and longterm outcome. To name selected examples, patients with a good prognosis are those with functional inactivation of the core binding factors (CBFs): AML1 and CBF. These cases include patients with AML and t(8;21) (q22;q22) or inv(16) (p13;q22), two of the most frequent recurrent cytogenetic abnormalities in de novo AML in younger patients. Poor-risk cytogenetics have a loss of all or part of chromosome 5 or 7, translocations involving 11q23, or abnormalities of chromosome 3. A model of a “two-hit-hypothesis” for the AML phenotype by so-called class I and II mutations has been established. It describes the cooperativity of activating mutations in FLT3 (Fms-like tyrosine kinase 3) (=class I) and gene rearrangements involving hematopoietic transcription factors (=class II). The expression of both classes may result in the AML phenotype. FLT3 mutations can appear in all subtypes of AML and with the majority of known chromosomal translocations associated with AML. In this hypothesis, FLT3 mutations serve as exemplary of class I mutations that, alone, confer a proliferative and survival advantage to hematopoietic progenitors but do not affect cell differentiation (Crowther *etal*, 2008).

2.1.3.3 Physical and Chemical Factors:

A variety of environmental and chemical exposures are assumed to be associated with a variably elevated risk of developing AML in adults. A selection of hazards will be mentioned here. Exposure to ionizing radiation is linked to AML. Among survivors of the atomic bomb explosions in Japan, an increased incidence of AML was observed with a

peak at 5–7 years after exposure. Also, therapeutic radiation has been found to increase the risk of secondary AML. Chemotherapeutic agents, such as alkylating agents and topoisomerase II inhibitors, have been reported to increase the incidence of AML and will be discussed in detail below. A number of other substances (therapeutic and occupational) have been linked to an increased risk of AML. Chronic exposure to certain chemicals clearly shows an increased risk for the development of AML. Benzene is the best studied and widely used potentially leukemogenic agent. Persons exposed to embalming fluids, ethylene oxides, and herbicides also appear to be at increased risk. Furthermore, smoking has been discussed to be associated with an increased risk of developing AML (particularly of FAB subtype M2), especially in those aged 60–75.

Viruses – particularly RNA retroviruses – have been found to cause many neoplasms in experimental animal models, including leukemia. As of now, a clear retroviral cause for AML in humans has not been identified even though an association between the exposure to certain viruses and the development of AML has been suggested. Parvovirus B19 could thus play a role in the pathogenesis of AML. It has so far not been demonstrated, however, that simple infection with either a RNA- or DNA-based virus alone is a cause of AML (Crowther *etal*,2008).

2.1.3.4 Secondary AML:

As mentioned, the cause of the disease is unknown for most patients with acute myeloid leukemia. “The true secondary AML” has been recommended to be referred to patients who have a clear clinical history of prior myelodysplastic syndrome (MDS), myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents; it is thus a rather broad category. Secondary leukemias are in more than 90% of myeloid origin. Patients have a particularly poor outcome, with a lower incidence of achieving complete remission and shorter duration of survival than for patients with *de novo* AML. Treatment-related secondary leukemia was first observed in survivors of successfully treated Hodgkin’s disease. Later on, survivors of ALL and other disease entities such as ovarian or breast cancer and multiple myeloma were included. The development of secondary AML shows a maximum in the 5–10 years following therapy. The distinct pattern of cytogenetic and

genetic abnormalities in secondary or treatment-related AML is worthy of notice. AML arises after previous therapy for other malignancies in a subset of 10–20% of patients. The risk of therapy-related AML after intensive chemotherapy may be increased to more than 100 times. Specific cytogenetic abnormalities currently serve as the most important factor in distinguishing differences in AML biology, response to treatment and prognosis. The different abnormalities result in gene rearrangements that may reflect the etiology and pathogenesis of the disease. Treatment-related or secondary leukemia are examples in which genetic aberrations provide information on its specific etiology. In understanding the mechanisms associated with the development of secondary AML, general facts about the possible etiology of leukemia can be elucidated. Cytogenetic changes that can be related to previous exposure to different chemically well-defined cytostatic agents with a known mechanism of action. Among those are for alkylating agents: deletions or loss of 7q or monosomy 7 with normal chromosome 5, and deletions or loss of 5q or monosomy 5. For epipodophyllotoxins, balanced translocations to chromosome bands 11q23, primarily in children, have been described. Topoisomerase II inhibitors have been linked to t(8;21), inv(16). Topoisomerase II inhibitors, anthracyclines, mitoxantrone, as well as radiotherapy may be associated with therapy-related acute prolymphocytic leukemia with t(15;17) and chimeric rearrangements between PML and RARA genes as well as different translocations to chromosome bands 11q15 and chimeric rearrangement between the NUP98 gene and its partner genes. Another subgroup includes 10–15% of all patients with secondary AML, with normal karyotype or various chromosome aberrations uncharacteristic of t-AML or at least not identified as such as of now. It is to be expected that in the future, many more genetic and epigenetic changes may be discovered. As of now, methylation of the p15 promoter is the only abnormality observed in a high percentage of patients with AML, especially in patients with secondary AML. In current times there is a rapid gain in insight regarding epi-/genetic changes associated with the development of hematological malignancies like AML. It can be hoped for that many epidemiological and etiological findings may be explained and the development of new specific treatment strategies can further be enhanced on this basis (Estey *et al*, 2008).

2.1.4 Clinical Features:

All of the signs and symptoms that present so abruptly in patients with AML are caused by the infiltration of the bone marrow with leukemic cells and the resulting failure of normal hematopoiesis. These malignant leukemic cells that invade the bone marrow are dysfunctional, and without the normal hematopoietic elements, the patient is at risk for developing life-threatening complications of anemia, infection due to functional neutropenia, and hemorrhage due to thrombocytopenia. Fatigue and weakness are the most common complaints that reflect the development of anemia. Pallor, dyspnea on exertion, heart palpitations, and a general loss of well-being has been described. Fever is present in about 15% to 20% of patients and may be the result of bacterial, fungal, and, less frequently, viral infections, or from the leukemic burden of cells on tissues and organs. Easy bruising, petechiae, and mucosal bleeding may be found due to thrombocytopenia. Other more severe symptoms related to decreased platelet counts that occur less commonly are gastrointestinal or genitourinary tract and central nervous system (CNS) bleeding. CNS infiltration with high numbers of leukemic cells has been reported in 5% to 20% of children and approximately 15% of adults with AML. Headache, blindness, and other neurological complications are indications of meningeal involvement. Leukemic blast cells circulate through the peripheral blood and may invade any tissue. Extramedullary hematopoiesis is common in monocytic or myelomonocytic leukemias. Organs that were active in fetal hematopoiesis may be reactivated to again produce cells when stressed by the poor performance of the overburdened leukemic bone marrow. Hepatosplenomegaly or lymphadenopathy may occur but is not as prominent as that seen in the chronic leukemias. Skin infiltration is very characteristic in monocytic leukemias, particularly gum infiltration, which is termed gingival hyperplasia (Cisela, 2007)

2.1.5 Classification:

Classification of AML For much of the twentieth century classification of AML was based on how leukemic blasts, the predominant cell in the disease process, recapitulate normal Hematopoiesis. Diagnosis and Classification of the Acute Myeloid Leukemia (with

Discussion of the Role of the Myelodysplastic Syndromes in AML Pathogenesis) David Head, Mary Ann Thompson *et al.* Are blasts in a given case myeloblasts, monoblasts, megakaryoblasts, etc., and are they un-, minimally, or moderately differentiated. This approach was formalized by the FAB working group in a series of papers beginning in 1976, allowing analysis of its relevance. As specified in the table, M0 designates AML with minimal morphologic or cytochemical differentiation, M1–2 AML with minimal or moderate granulocytic differentiation, M3 acute promyelocytic leukemia (APL), M4 AML with mixed myelomonocytic differentiation, M5a and M5b monoblastic leukemia with minimal or moderate differentiation, M6a myeloid leukemia with dysplastic background erythropoiesis, M6b acute erythroblastic leukemia, and M7 acute megakaryoblastic leukemia. Unfortunately, subsequent analyses showed a general lack of clinical and biological relevance to this long standing approach, e.g., failure to predict outcome, partial and imprecise correlation with cytogenetics, failure to predict expression of the membrane pump MDR1, and failure to predict clonality of presentation and remission hematopoiesis. The approach remains a useful shorthand descriptor of myeloblast morphology, but should not be used alone as a classification of AML. Instead, what has become apparent is that the phenotype of AML is divisible into two approximately equal broad pathogenetic groups of disease, which the authors designate DN (*de novo*)-AML and MDR (myelodysplasia-related)-AML. The median age of AML is in the mid-60s in the US and Western Europe, with an exponential age incidence curve with advancing age, most noticeable after age 50. The age incidence of MDR-AML substantially mimics this exponential curve, while the age incidence of DN-AML is approximately flat for population at risk throughout life. Distinction of DN- versus MDR-AML is very important, as the latter group has MDS-like background hematopoiesis, with poor marrow reserve and the probability of reversion to clonal hematopoiesis (i.e., MDS) during “remission.” MDRAML also has a high frequency of resistance to chemotherapy at presentation. The WHO (World Health Organization) Classification of AML (2001) appropriately adopted this distinction in its first two categories of AML. (It should be noted that in its fourth category the WHO Classification confusingly retained a modified FAB AML classification that redundantly overlaps almost

completely the first two categories; comments above about the FAB classification apply equally to this fourth category of the WHO classification.). Morphologic (modified FAB) description of acute myeloid leukemia: M0 AML with no Romanowsky or cytochemical evidence of differentiation, M1 Myeloblastic leukemia with little maturation, M2 Myeloblastic leukemia with maturation M3 Acute promyelocytic leukemia (APL) (M3h APL, hypergranular variant, M3v APL, microgranular variant), M4 Acute myelomonocytic leukemia (AMML) (M4eo AMML with dysplastic marrow eosinophils), M5 Acute monoblastic leukemia (AMoL) (M5a AMoL, poorly differentiated, M5b AMoL, differentiated) M6 “Erythroleukemia”* (M6a AML with erythroid dysplasia, M6b Erythroleukemia) M7 Acute megakaryoblastic leukemia (AMkL) (Estey *et al*, 2008).

2.1.5.1 De Novo AML:

DN-AML is the more common AML of children and young to middle age adults, with a median age in the 30s corresponding approximately to the median age of the population. DN-AML occurs with approximately flat incidence throughout life. This flat incidence curve suggests a single rate limiting pathogenic step in development of disease. To the extent that the molecular pathogenesis of DN-AML has been clarified, cases are characterized by one of a series of recurring genetic abnormalities that block differentiation of hematopoietic precursors. However, as suggest by Gilliland, a block of differentiation alone is insufficient to create the phenotype of AML, and a drive to proliferate, probably coupled with inhibition of apoptosis, is also requisite for the acute leukemia phenotype of DN-AML. Such additional genetic damage has been identified in a large percentage of DN-AML cases. The most common abnormality of this type is mutation of FLT3, a membrane receptor tyrosine kinase that normally in the presence of its ligand drives proliferation and inhibits apoptosis of hematopoietic progenitors. FLT3 mutations in AML constitutively activate its kinase activity in affected hematopoietic precursors. Apparently these effects, coupled with a block of differentiation, combine to generate the AML phenotype. Other mutations have been described that drive proliferation in DN-AML, e.g., activating RAS, cKit, and CSF-1 receptor mutations. Both the proliferative drive mutations and the block of differentiation mutations are mutually

exclusive of other mutations in each group, suggesting the members of each group contribute the same biologic component to transformation. It should be noted that the two sets of genetic damage appear to occur substantially independently of one another (e.g., FLT3 mutations have been described with each common recurring translocation in DN-AML). It has been suggested that multiple additional biologic events are necessary to transform normal cells into solid tumor malignancies, but it is unclear if this information is applicable to acute leukemia, and if so whether additional events need be genetic versus epigenetic or physiologic. Virtually all recurring genetic abnormalities identified to date in DN-AML either block differentiation or drive proliferation and inhibit apoptosis. In the WHO classification of AML, DNAML falls under AML with Recurrent Cytogenetic abnormalities with sub classification based on the common recurring cytogenetic translocations of DN-AML. A limitation of this approach can be seen from the previous paragraph. The classification includes only one of the two known genetic elements required for the leukemic phenotype, yet both elements have biologic. · Classification of AML biologic studies, and monitoring of disease .DN-AML has polyclonal background hematopoiesis at presentation, and remissions that restore normal polyclonal hematopoiesis with production of normal peripheral blood counts .Both at presentation and in remission, background hematopoiesis lacks the dysplastic morphology characteristic of MDS. In some instances one of the genetic events required for transformation may remain present in some precursor cells in remission, e.g., persistent positivity for the fusion message *AML1/ETO* during prolonged remission in some patients with AML with t(8;21). The clinical significance of this finding is not completely certain, but suc0h patients may remain in stable remission indefinitely; it should be noted that the fusion protein product in this setting usually appears to block differentiation of affected cells, rather than driving proliferation, supporting the concept that a block of differentiation alone is insufficient for leukemogenesis. As a group DN-AML tends to be responsive to cytotoxic chemotherapy, and that failing, most patients are potential candidates for allogeneic stem cell transplantation due to young median age. Therapy-related AML mimicking DN-AML occurs in at least two clinical settings. The more common setting is following epipodophyllotoxin chemotherapy, with 11q23/*MLL* translocations or less

frequently 21q22/*AML1* translocations. Less commonly, all of the common recurring balanced translocations of DNAML occur with increased frequency following complex multiagent chemotherapy. These two settings appear to be iatrogenic models of DN-AML pathogenesis. (Estey *et al*, 2008).

2.1.5.2 Myelodysplasia-Related AML:

MDR-AML is the common AML of the elderly, comprising the majority of AML cases beyond age 60, with a median age in the 70s. While it does occur in children and young adults, its incidence for population at risk comprises an exponential curve with progressive age, accounting for this feature of the incidence curve of AML as a whole. The exponential curve suggests several random events are required for generation of this set of AML. MDR-AML is characterized by a series of cytogenetic changes shared with MDS. Despite extensive efforts, the genes affected and the biologic impact of the cytogenetic changes common to MDR-AML and MDS (e.g., -7, 5q-, +8, 20q-, +21) remain unclear. These cytogenetic changes appear to relate to progression of MDS, rather than its initiation, as they are absent in 60% of MDS at presentation, and all of the common cytogenetic changes may appear as clonally selected changes after initial presentation of MDS. Presence of cytogenetic changes characteristic of DN-AML precludes diagnosis of MDRAML. In parallel with DN-AML, the limited data available appear to support the requirement for both a block of differentiation and a drive to proliferate (coupled with inhibition of apoptosis) in the pathogenesis of MDR-AML. Activating RAS, FLT3, and CFMS mutations, all of which drive proliferation through the RAS pathway, are found in some cases of MDR-AML. As in DN-AML, these mutations are mutually exclusive of one another, suggesting they each contribute the same function to transformation. Deregulated expression of *EVI-1*, which blocks the differentiating effect on hematopoietic precursors of GATA-1, also precedes transformation of MDS to AML in some cases, and inactivating mutations of GATA-1 have been described in MDR-AML occurring in Down syndrome children. Thus, available evidence suggests two things. First, acquisition of the MDR-AML phenotype requires a parallel set of genetic damage to that required for the DN AML phenotype, both resulting in the generic phenotype of AML. Second, in MDR-AML

acquisition of this genetic damage appears to derive from the mutator phenotype of MDS, while in DNAML the flat incidence curve suggests random genetic damage as the cause. In the WHO classification, MDR-AML is categorized as AML with Multilineage Dysplasia. Acceptance of this basic distinction of MDR-AML and DN-AML. (Estey *et al*, 2008).

2.1.6 WHO AML with Recurrent Cytogenetic Abnormalities:

AML with recurrent cytogenetic abnormalities AML with t(8;21)(q22;q22), (AML1/ETO) AML with inv(16)(p13;q22) or t(16;16)(p13;q22),(CBF/MYH11)AML with t(15;17)(q22;q12), (PML/RAR) (APL) AML with 11q23 (MLL) abnormalities AML with multilineage dysplasia With prior myelodysplastic syndrome without prior myelodysplastic syndrome AML and MDS,therapy related alkylating agent type (Estey *et al* ,2008).

2.1.7 Pathogenesis Signs and Symptoms:

2.1.7.1 Bone Marrow Infiltration:

Neutropenia, Anemia and Thrombocytopenia.

2.1.7.2 Medullary Infiltration:

Marrow, Extramedullary *Infiltration*, Liver, spleen, lymph nodes, thymus. Central nervous system, Gums, mouth, Skin, Fever, infection ,Pallor, dyspnea, lethargy ,Bleeding, petechial, ecchymosis, intracranial hematoma and gastrointestinal or conjunctiva hemorrhage (rare) Bone pain and tenderness, limp, arthralgia ,Organomegaly Neurological complications including dizziness, headache, vomiting, alteration of mental function ,Gingival bleeding and hypertrophy. Myeloblasts may be distinguished from lymphoblasts by three distinct ways: presence of Auer rods, reactivity with cytochemical stains, or reactivity with cell surface markers (for example, clusters of differentiation [CD] groups CD13, CD33) on blasts with specific monoclonal antibodies. The morphology of blasts can often be determined by an experienced morphologist; however, other supporting tests are always needed to confirm the initial designation. The features that can be used to differentiate a myeloblast from a lymphoblast.The chromatin material of a myeloblast is usually much finer than that of a lymphoblast. A myeloblast often has more

cytoplasm than a lymphoblast. Both size of the blast and number of nucleoli may not be helpful characteristics. Although a myeloblast is usually larger than a lymphoblast, sufficient variations are seen that this is not the best factor to consider. Along the same lines, the number of nucleoli that can be seen in a myeloblast is one to four, and a lymphoblast one or two, so when deciding lineage on a blast with two obvious nucleoli, either choice would be acceptable. Therefore, of the characteristic features the most helpful is usually the chromatin staining pattern. As mentioned previously, other methods besides morphological examination must be used to confirm the type of blasts present, and often to quantify the number of blasts, particularly when two blast populations coexist in significant amounts in the leukemic bone marrow. Other studies that can be used to diagnose the acute leukemia include chromosome analysis, molecular genetic studies, DNA flow cytometer, and electron microscopy. From 5% to 10% of the AMLs have a preleukemic.

Presentation termed “myelodysplastic syndrome.” These patients are usually over the age of 50 and have anemia, thrombocytopenia, and monocytosis but with bone marrow blast percentages of less than 20% (Cisela, 2007).

2.1.8 Laboratory Findings:

2.1.8.1 Peripheral Blood and Bone Marrow Findings:

The CBC and examination of peripheral blood smear are the first step in the laboratory diagnosis of leukemia. Blood cell counts are variable in patients with AML. The WBC may be normal, increased, or decreased. It is markedly elevated, over $100 \times 10^9/L$ cells in less than 20% of cases. Conversely, the WBC is less than $5.0 \times 10^9/L$ with an absolute neutrophil count of less than $1.0 \times 10^9/L$ in about half the patients at the time of diagnosis. Blasts are usually seen on the peripheral smear examination, but in leukopenia patients, the numbers may be few and require a diligent search to uncover. Cytoplasmic inclusions known as Auer rods often present in a small percentage of the myeloblasts, monoblasts, or promyelocytes present in the various subtypes of AML. Auer rods are elliptical, spindle-like inclusions composed of azurophilic granules. Nucleated red blood cells may be present, as well as

myelodysplastic features, including pseudo-hypossegmentation (pseudo Pelger-Huët cells) or hypersegmentation of the neutrophils, and hypogranulation. Anemia is a very common feature due to inadequate production of normal red cells. The reticulocyte count is usually normal or decreased. Red cell anisopoikilocytosis is mildly abnormal, with few poikilocytes present. Thrombocytopenia, which can be severe, is almost always a feature at diagnosis. Giant platelets and agranular platelets may be seen. Disseminated intravascular coagulation (DIC) is most commonly associated with one of the types of AMLs known as acute promyelocytic leukemia. The DIC is caused by the release of tissue factor–like procoagulants from the azurophilic granules of the neoplastic promyelocytes, which in turn activate coagulation and further consume platelets, leading to dangerous bleeding diathesis. Before treatment, serum uric acid and lactic dehydrogenase (LDH) levels often are mild or moderately increased (Bain, 2004)

The hallmark feature of acute leukemia is always a hypercellular bone marrow, with 20% to 90% leukemic blasts at diagnosis or during relapse. The blast population grows indiscriminately as these cells have only limited differentiation capability and are frozen in the earliest stage of development. The lineage of blasts that predominate depends on the specific type of acute leukemia. The most current classification for hematological and lymphoid tumors published by the World Health Organization (WHO) recommends that the requisite blast percentage for a diagnosis of acute myeloid leukemia be greater than or equal to 20% myeloblasts in the blood or marrow. When performing a peripheral blood smear on a patient with a suspected diagnosis of leukemia, at least 200 WBCs should be classified. It is recommended that the blast percentage in the bone marrow be derived from a 500-cell differential count. If the WBC is less than $2.0 \times 10^9/L$, buffy coat smears should be prepared for differential counting (Cisela, 2007).

2.1.8.2 Cytochemical Stains:

Cytochemical stains are very helpful in the diagnosis and classification of acute leukemia. These stains are usually performed on bone marrow smears but may also be done on peripheral smears or bone marrow touch preps. The special stains are used to identify enzymes or lipids within the *blast* population of cells—hence, the reaction in mature cells is not of

importance. The positive reactions that occur will be associated with a particular lineage, and with some of the stains (e.g., myeloperoxidase [MPO] and Sudan Black B [SBB]), the fine or coarse staining intensity is an indication of the lineage of blast cells. All of the cytochemical stains described below are negative in lymphoid cells (with rare exceptions), so a positive result with any of these will most often rule out acute lymphoblastic leukemia (Cisela, 2007).

2.1.8.2.1 Myeloperoxidase:

Primary granules of myeloid cells contain peroxidase. The granules are found in the late myeloblast and exist throughout all the myeloid maturation stages. Because primary granules are absent in myeloblasts, there is limited MPO activity in early myeloblasts; however, those blasts that are closer to maturing to the promyelocyte stage will stain positive. Promyelocytes, myelocytes, metamyelocytes, and band and segmented neutrophils will stain strongly positive, indicated by the presence of blue-black granules. Monocytic granules stain faintly positive. Because lymphoid cells, nucleated red cells, and megakaryoblasts lack this enzyme, they will stain negative. This negative reaction is useful in initially differentiating ALL from AML. Eosinophils will also stain positive for MPO. Auer rods are strongly positive for peroxidase. The enzymatic activity in blood smears will fade over time, so slides should not be held for staining for longer than 3 weeks. The MPO stain will be positive in acute myeloid leukemia (3% positive), acute promyelocytic leukemia (90% to 100% positive), acute myelomonocytic leukemia (including AMML with abnormal eosinophils, AMML Eo [variable, 3% positive]), acute monoblastic and monocytic leukemia (variable), and in the myeloblasts present in acute erythroid leukemia (myeloblasts, 3% positive) (Cisela, 2007).

2.1.8.2.2 Sudan Black B:

Phospholipids and other intracellular lipids are stained by SBB. Phospholipids are found in the primary (nonspecific) and secondary (specific) granules of neutrophilic cells and eosinophils, and in smaller quantities in monocytes and macrophages. The stain will be negative in lymphocytes, although rarely the azurophilic granules of lymphoblasts may demonstrate positivity. The SBB pattern of staining mimics the

MPO stain in that it is very sensitive for granulocyte precursors, increases in staining intensity with the later stages of granulocytic maturation, and stains weakly positive with monocytic cells. Thus, this stain can also be used to differentiate AML from ALL. A distinct advantage of the SBB over the MPO stain is that the SBB-stained slides are stable for a longer period of time (Cisela, 2007).

2.1.8.2.3 Specific Esterase (Naphthol AS-D Chloracetate Esterase):

The specific esterase enzyme is present in the primary granules of myeloid cells. Accordingly, myeloblasts and other neutrophilic cells in AML will stain positive. This stain will also be positive in basophils and mast cells but is negative in eosinophils, monocytes, and lymphocytes. The specific esterase enzymatic reaction is stable in paraffin-embedded tissue sections, making this an extremely useful stain for identifying cells of myeloid lineage in extramedullary myeloid tumors (Cisela,2007).

2.1.8.2.4 Nonspecific Esterase (Alpha-Naphthyl Butyrate or Alpha-Naphthyl Acetate Esterase):

These stains are used to identify monocytic cells and will stain negative with granulocytes. Different substrates are available, with alpha-naphthyl butyrate stain considered more specific and alpha-naphthyl acetate stain being more sensitive. Many cells in addition to monocytes will stain positive (macrophages, histiocytes, megakaryoblasts, and some carcinomas), so the sodium fluoride inhibition step is used to differentiate the positivity. With this step, following initial staining, the NSE activity of monocytes and macrophages is Cytochemical Reaction Cellular Element Stained Blasts Identified, Myeloperoxidase (MPO), Sudan Black B (SBB) ,Specific esterase ,Nonspecific esterase (NSE) ,Terminal deoxynucleotidyl ,transferase (TdT) ,Periodic acid-Schiff ,Neutrophil primary granules ,Phospholipids ,Cellular enzyme ,Intranuclear enzyme (Cisela ,2007).

2.1.8.3 Flow Cytometry:

Immunophenotype:

Immunophenotyping can help to classify the clone of leukemic blasts by using monoclonal antibodies directed against cell surface markers. The

specific lineage and stage of maturation can be tagged, and this information then is used to indicate appropriate therapy and can be correlated to prognosis. Immunophenotyping can be done by flow cytometry or by immunohistochemistry methods. Multiple antigens can be detected simultaneously on a single cell using flow cytometry. A selected panel of monoclonal antibodies (Cisela ,2007)

An Immunophenotyping blast by flow cytometry has become standard for diagnosis of the acute leukemias, in particular for separation of AML and ALL, as well as for recognition of myeloblastic, monoblastic or megakaryoblastic differentiation in leukemic blasts. Variations in details of flow cytometry are beyond the scope of this chapter, but some comments with respect to its application to AML are appropriate. A standard flow cytometric approach for acute leukemia is to use a combination of CD45, side scatter, and forward scatter to recognize an abnormal population consistent with blasts, with blasts usually having reduced CD45 and low side scatter. This population is then characterized with a series of antibodies directed against surface antigens present on immature hematopoietic cells to separate B or T lymphoblasts from blasts with myeloid differentiation, and to delineate lineage commitment of the myeloid blasts. It should be noted again that lineage commitment is not a satisfactory approach for sub classification of AML, but it is useful for confirming diagnosis of AML versus ALL. Commonly used surface antigens for this purpose are: for myeloid differentiation CD13, CD33, CD117, CD16, CD64, CD14, CD11b, CD4(2-), and CD61; for B-lymphoid differentiation CD19, CD79a,CD22, CD20, CD10, and TdT; and for T-lymphoid differentiation CD2, CD7, CD4, CD8, CD1a, and TdT. None of these antigens singly are lineage-specific, but rather lineage-associated, but unambiguous sets of lineage-associated antigen positivity reliably indicate lineage commitment of blasts. Myeloid blasts can be further characterized as having myeloblastic, monoblastic (CD4+, CD14+, CD64+), or megakaryoblastic (CD61+) differentiation. There are two general approaches to resolve ambiguous cases. One approach, proposed by the European Group for Immunologic Classification of Leukaemia (EGIL), is a scoring system that applies variable weight to surface antigens, plus several cytoplasmic antigens (cytoplasmic [c]CD79a, cCD22, cCD3, and (cMPO). This approach requires a score of greater than 2 for confirmation of lineage

differentiation. The second approach treats only a limited panel of cytoplasmic markers as definitive for lineage differentiation of blasts: cCD79a or cCD22 for B differentiation, cCD3 for T differentiation, cMPO for myeloid differentiation, and cCD61 for megakaryoblastic differentiation. Frequent cases of acute leukemia demonstrate “aberrant” expression of antigens typically associated with another lineage of differentiation. This expression has little bearing on prognosis of a given case, and does not indicate bilineage differentiation in most such cases of acute leukemia. Such expression may provide a useful marker of leukemic blasts for monitoring treatment of such cases with flowcytometry for low-level residual disease. A small percentage of cases of acute leukemia do demonstrate dual lineage differentiation of blasts, either B-myeloid or T-myeloid. Many such cases **demonstrate** dual morphology with small lymphoblasts and larger myeloblasts (often more apparent in thicker areas of smears), often with granular differentiation or Auer rods in myeloblasts. In addition, cytochemical stains may demonstrate dual populations, with Sudan black or myeloperoxidase cytochemical positivity in the larger blasts. Confirmation of such dual differentiation requires flow cytometric demonstration of expression of multiple myeloid and either B- or T-lymphoid lineage-associated antigens or lineage-specific cytoplasmic markers. there are two general approaches for confirmation of bilineage commitment of blasts, either use of the EGIL scoring system or demonstration of confirmatory cytoplasmic antigen positivity. In this situation cytochemical myeloperoxidase or Sudan black positivity in leukemic blasts can supplement flow cytometric demonstration of myeloid differentiation. Finally, some have recommended the terminology of bilineal acute leukemia for cases with two distinct populations of blasts, versus biphenotypic for cases with expression of markers of two distinct lineages of differentiation on the same blasts. In both settings both populations of blasts usually have a uniform set of genetic abnormalities. Only very rarely are two completely unique sets of genetic abnormalities present in distinct blast populations in acute leukemia, suggesting simultaneous occurrence of two unrelated diseases.

There are several pitfalls and caveats in evaluation of leukemic blasts with flow cytometry. With standard flow cytometric CD45/side scatter gating for a blast population, the position of leukemic promyelocytes

tends to merge with the normal granulocyte population, compromising recognition and separation of the two populations in APL. Monoblasts and promonocytes tend to merge with normal monocytes with the same gating, and this separation is further complicated by the presence of few distinguishing cytometric markers between the two populations. Therefore, morphologic correlation is essential when evaluating monoblastic leukemia. Hypogranular granulocytes in MDS and MDR-AML tend to merge with the blast gate, because of decreased side scatter, causing overestimation of the blast percentage in such cases. Elimination of erythroid precursors in processing the sample before flow analysis may falsely elevate the blast percentage by reducing the denominator in the sample. Despite these limitations, flow cytometry has become an indispensable tool for rapid and efficient diagnosis of acute leukemia. Flow cytometric analysis can be adapted to monitoring minimal residual disease during treatment of AML, with sensitivity of approximately 1 cell in 10³⁻⁴, if an informative phenotype differing from normal populations is identified. In this capacity this technology may be superior to other approaches because of its speed and cost, but may require greater than the current standard 4-color flow cytometry to be informative and practical in most cases. A number of reports indicate that demonstration by flow cytometry of aberrant expression, or aberrant timing of expression, of antigens in myeloid progenitors may be a useful adjunct for diagnosis of MDS. Such testing is not yet widely used for this purpose in clinical practice, in part because it requires more extensive antigen panels and/or greater than the usual current 4-color flow cytometry for implementation. However, with technical improvements in cytometric equipment such testing could become a valuable adjunct for confirmation of diagnosis of MDS, and similar testing of background hematopoiesis could potentially be used in differential diagnosis of MDR- and DN-AML (Estey *et al*, 2008).

Acute Myeloid Leukaemia (AML) accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. Immunophenotyping has become extremely important not only in diagnosis and subclassification of AML but also in the detection of the minimal residual disease. Immunophenotypic pattern of AML in Sudanese patients have not been addressed before. This study was conducted to characterize immunophenotypic patterns of AML in

Sudanese patients. Multiparameter flow cytometry and CD45/SSC gating were used to analyze the surface and cytoplasmic antigen expressions in 106 cases of AML during the period mid2010 to mid2011 at Radioisotope Centre Khartoum (RICK). The following antigens: CD45, HLA-DR, CD34, CD117, CD13, CD33, CD19, CD7, cytoplasmic markers (CD3, CD79a, MPO), CD11c, CD14, CD64, CD42a, CD41 and CD61 were used. Results: Almost all AML blasts were expressing CD45 with no significant differences between the subtypes. CD34 have different expressions in AML subtypes. CD13 and CD33 were also studied among the blast population having mean positivity of 51.5% and 49.8% respectively in all AML subtypes collectively. CD33 was found to have higher positivity among AML-M4 and AML-M5 with mean positivity of 75.9% and 76.6% respectively. CD13 and CD33 had no correlation for all AML subtypes except for AML-M5 with very strong negative correlation($r=-0.913$). Apparent expression of CD7 and CD19 were expressed in 45.1% and 13.6% of all cases respectively. CD7 was mostly expressed in AML-M2 and AML-M3 (75%) and least in AML-M5, while CD19 was only expressed in cases of AML-M0 and AML-M7. Conclusion: Flowcytometric analysis of acute leukemia by combining the patterns and intensity of antigen expression improved the diagnosis of AML in our centre. Immunophenotyping results and FAB classification of our AML patients were comparable to international published studies (Osman *et al*,2015)

2.1.8.4 Cytogenetics and Molecular Genetics:

Karyotype analysis remains indispensable as a diagnostic tool for acute leukemia, due to its ability to screen the entire complement of chromosomes for large scale abnormalities. In the majority of childhood acute leukemia and 54–78% of adult AML, cytogenetic abnormalities are detected on karyotype analysis of blood or marrow. Cytogenetic studies at diagnosis help separate DN- and MDR-AML, and are currently one of the most valuable prognostic indicators for AML, separating patients into favorable, intermediate, or unfavorable clinical risk groups. The translocations of AML with Recurrent Cytogenetic Abnormalities (WHO) each have prognostic significance: $t(8;21)$, $inv(16)$, and $t(15;17)$ confer favorable risk, 11q23 abnormalities intermediate risk. Of the karyotypic changes associated with MDS or MDR-AML, -5 and -7 are in

the adverse risk group, while +8 and +21 are intermediate risk. A second reason that cytogenetic studies should be done on all new AML cases is that treatment options may vary depending on cytogenetic results, particularly with t(15;17) (APL) and t(9;22) (which may indicate myeloid blast crisis of CML). Molecular diagnostic tools such as fluorescent in situ hybridization (FISH) However, there is a role for these directed tests to increase detection sensitivity for the common translocations, additions, and deletions of AML, to confirm translocation breakpoints detected by karyotyping, and to detect cytogenetically silent genetic abnormalities [e.g., t(12;21) of ALL, FLT3 internal tandem repeats, *C/EBP* mutations]. In addition to karyotyping, most academic centers use FISH or RT-PCR to test for *PML/RAR*, *CBF/MYH11*, *AML1/ETO*, and *MLL* disruption, and of-ten use FISH to search for -7, 5q-, and +8. Although these examples are usually detected by classic karyotype analysis, poor viability and inability to obtain metaphase spreads may compromise karyotype analysis, and cryptic insertional translocations occur that cannot be detected by karyotype analysis. An additional advantage of FISH is that it can be performed on paraffin sections of nondecalcified tissue, such as bone marrow particle sections. Thus, in AML cases with a negative karyotype FISH or RT-PCR is recommended for some common abnormalities. For the more common translocations, the following are estimates of the percentage of cases missed if only karyotype analysis is performed: *AML1/ETO*, 6%; *CBF/MYH11*, 4%; *PML/RAR*, 15%. These cases include those where karyotype analysis was unsuccessful, as well as cases with cryptic translocations. With the additional methods of testing the overall incidence of the major recurring translocations in AML in the US and western Europe, from age 2 to middle age, are: *MLL*(11q23): 3–7%, *AML1/ETO*: 14%, *PML/RAR*: 10–15%, *CBF/MYH11*: 10%. (It should be noted that there is heterogeneous distribution of some of these translocations (Drew, 2003))

2.1.8.4.1 Specific Recurring Genetic Abnormalities of AML:

A variety of specific genetic abnormalities have been described in patients with AML, some of which appear to block differentiation, while others drive proliferation and inhibit apoptosis. Abnormalities of each functional group appear to be mutually exclusive of other abnormalities of the same group, but as discussed previously, abnormalities of both

groups appear necessary to create the acute leukemia phenotype. As stated previously, despite extensive efforts specific genetic abnormalities caused by the major chromosomal abnormalities typical of MDS and MDR-AML (i.e., -7, 5q-, +8, 20q-, +21, etc.) that contribute to disease pathogenesis remain unknown (Estey *et al*,2008).

2.1.8.4.1.1 Retinoic Acid Receptor Translocations:

The successful treatment of acute promyelocytic leukemia with retinoic acid (RA) is a stunning example of the potential power of molecular medicine. As the association between t(15;17) (q22;q21) and APL was known, the report that oral all trans-retinoic acid (ATRA) induced complete remission in APL led quickly to demonstration that t(15;17) involves the retinoic acid receptor gene (*RAR*) at chromosome 17q21 (Estey *et al*,2008).

2.1.8.4.1.2 Core Binding Factor Mutations:

The core binding factor translocations include t(8;21)(q22;q22) which produces the AML1/ETO fusion protein and inv(16), which produces the CBF /MYH11 fusion protein. The murine counterpart of *AML1* (*RUNX1*, *CBF1*) was first described as part of the heterodimer core binding factor (CBF) which binds to a core enhancer sequence of the Molony leukemias virus LTR. Inversion(16)(p13;q2), and t(16;16) (p13;q22) disrupt the other component of CBF, non-DNA binding CBF . The core enhancer sequence is found in the promoter region of a number of human genes, including Bcell tyrosine kinase, T cell receptors and ,the cytokines IL3 and GM-CSF, and the granulocyte proteins MPO and neutrophil elastase .Core binding factor translocations appear to contribute a block of differentiation to leukemia pathogenesis. Core binding factor is essential for hematopoietic development. Knockout of either *AML1* or *CBF* in mice results in fetal death at E11.5–12.5 due to absence of fetal hematopoiesis. Transgenic experiments have demonstrated that AML1 is essential for development of hematopoietic stem cells in the aorta/gonadal/ mesodermal region, the source of definitive hematopoiesis. The essential role of AML1 in hematopoietic development appears to be through its function as a transcriptional activator. *AML1* is located on chromosome 21q22.3 and comprised of 12 exons over 260 kb of DNA, with 4 different transcripts by alternative

splicing. The runt homology domain (RHD) in the N-terminal portion of the protein, homologous to the *Drosophila runt* protein, is a sequence specific DNA binding domain. This domain is mutated in the uncommon autosomal dominant inheritance syndrome of familial platelet disorder with predisposition to AML (FPD-AML) and in AML associated with AML1 point mutations. CBF interacts via this domain and changes the conformation of AML1 to increase its DNA binding affinity. C-terminal to the RHD are potential MAP kinase phosphor-oid Leukemias ylation sites, followed by three activation domains, a nuclear matrix target signal, a dimerization domain, and sequences recognized by corepressor proteins (reviewed in). The *ETO* gene, cloned from the t(8;21) fusion, is the mammalian homologue of the *Drosophila nervy* gene. ETO is expressed in CD34+ progenitor cells but not in more differentiated hematopoietic cells. ETO does not appear to bind DNA specifically. However, it may act as a corepressor protein, as it associates with N-CoR and mSin3A, and directly binds to the class I HDACs (HDAC-1, HDAC-2, and HDAC-3). In t(8;21) the breakpoint in *AML1* is between exons 5 and 6, yielding a fusion protein with the N-terminal 177aa of AML1 including the DNA binding domain, but lacking the C-terminal activation domains, corepressor interaction sites, and nuclear localization signals of wild-type AML1. The breakpoint in the *ETO* gene occurs in the introns between the first two alternative exons of *ETO*, resulting in the inclusion of almost all of the coding region for ETO in the fusion transcript. The AML1/ETO protein binds to the same DNA sequence as AML1, heterodimerizes with CBF, and acts as a dominant negative inhibitor of wild-type AML1. AML1/ETO can also function as an active transcriptional repressor through its association with HDACs (via ETO). Targets of AML1/ETO repression are presumed to include genes important for granulocyte differentiation. In addition, AML1/ETO may indirectly repress p53 by repressing p19ARF, an antagonist of the p53 inhibitor MDM2; thus reduced p53 checkpoint control might contribute to t(8;21) leukemogenesis. Since observed breakpoints in *AML1* and *ETO* are clustered, RT-PCR using single *AML1* and *ETO* primers should detect the translocation in all cases. In addition, an *AML1/ETO* dual color, dual fusion probe is available for FISH. Other mutations involving AML1 have been described in AML and MDS. The uncommon t(3;21) involves the *AML1* gene and has been described in the setting of AML and MDS

following alkylating agent chemotherapy. In addition, several mutations of *AML1* have been identified in FPD-AML and in random cases of AML, including nonsense mutations and intragenic deletion of one allele of *AML1*. Finally, a minority of AML following epipodophyllotoxin chemotherapy are characterized by *AML1* translocations. AML with t(8;21) is a favorable prognostic subtype of AML, particularly with regimens containing highdose cytosine arabinoside. The majority of AML with t(8;21) demonstrates differentiation of blasts (M2 morphology) with cytoplasmic granules and Auer rods. In Romanowsky stained marrow smears maturing neutrophils from these patients may have characteristic salmon pink cytoplasm in the Golgi zone and pseudo Pelger-Huet nuclei. Flow cytometry often reveals aberrant expression of CD19 or CD56. Inv(16) and t(16;16) result in fusion of the first 165aa of CBF to the C-terminal coiled-coil region of MYH11]. The C-terminal region of MYH11 associates with mSin3a and HDAC8, contributing the transcriptional corepressor activity of the fusion protein. Presumably CBF /MYH11, which cannot bind DNA on its own, interacts with AML1 to form a transcriptional repressor complex. Inversion 16 AML often associates with myelomonocytic leukemia with dysplastic eosinophils in marrow with purple as well as orange granules (M4Eo) [3, 87]. These abnormal granules are evident especially in eosinophilic myelocytes. Cytogenetic detection of Inv(16) is subtle and may be missed with traditional standard Giemsa banding karyotype analysis. A common secondary abnormality, trisomy 22, or characteristic dysplastic eosinophils in smears, should prompt RTPCR for *CBF/MYH11*. Most *CBF* breakpoints occur in intron 5; breakpoints in 7 different exons have been reported in *MYH11*, but type A accounts for 90% of cases. Primers appropriate for these most common breakpoints are used for RT-PCR (Estey *et al* ,2008).

2.1.8.4.1.3 MLL Abnormalities:

The mixed lineage leukemia gene (*MLL*) (a.k.a. *HRX* or *ALL1*), a transcriptional activator frequently rearranged in infant leukemia, subtypes of therapy-related leukemia, and mixed lineage leukemia, maps to chromosome 11q23. In the *MLL* gene there are 11 sites similar to topoisomerase II consensus binding sites in breakpoint cluster areas. Infant leukemia with *MLL* translocations has a similar distribution of

breakpoints to therapy related disease, whereas sporadic cases have more random breakpoints. This observation has triggered speculation that in utero exposure to environmental topoisomerase II inhibitors such as flavonoids may have a role in the etiology of infant leukemia, although this remains unsubstantiated (Estey *et al*, 2008).

2.1.8.4.1.4 C/EBP:

C/CAAT enhancer binding protein (C/EBP) is a transcription factor that regulates granulocytic differentiation. The gene produces two proteins using alternative start sites. The larger and predominant 42 kDa protein consists of two N-terminal transactivating domains, with a C-terminal bZIP domain consisting of a basic (b) region that mediates DNA sequence binding and a leucine zipper (ZIP) domain that mediates dimerization. The shorter 30 kDa protein is transcribed from an alternative internal start site, and retains its bZIP domain but lacks the first transactivation domain; it thus retains the ability to dimerize with the long form, but inhibits transactivation by the dimer. The importance of C/EBP in granulocyte differentiation is supported by the lack of granulocyte differentiation in *CEBPA* knockout mice, while its conditional expression triggers granulocyte differentiation in bipotential precursors. C/EBP transactivates the genes for G-CSF and GM-CSF receptors, and several granulocyte-specific proteins. Cytogenetically silent mutations of *CEBPA* have been identified in about 8% of AML cases, and are of two types: C-terminal bZIP domain mutations and N-terminal truncating mutations that lead to enhanced production of the 30 kDa proteins. The former inhibit dimerization and DNA binding. The latter dimerize with the long form, but inhibit transactivation by the dimer, functioning in a dominant negative manner. *CEBPA* mutations are found predominantly in AML with differentiation (M2), similar to AML1 mutations, interesting in that C/EBP interacts with CBF to mediate AML differentiation. Some cases also have associated eosinophilia (M2Eo). Mutation of *CEBPA* has also been described in one family with a familial AML syndrome [109]. Approximately one third of AML with *CEBPA* mutations also have *FLT3* mutations. Data suggest that AML with *CEBPA* mutation and lacking *FLT3* mutation is favorable risk disease, remaining cases falling in the intermediate risk AML category (Estey *et al*, 2008).

2.1.8.4.1.5 GATA1:

GATA1 is a zinc finger transcription factor that regulates erythroid and megakaryocytic differentiation. Familial mis-sense mutations in *GATA1* results in a syndrome of dyserythropoietic anemia and thrombocytopenia, while conditional knockout of *GATA1* in megakaryocyte precursors in mice leads to thrombocytopenia and megakaryoblast proliferation. In acute megakaryoblastic leukemia (AMkL) that occurs in Down Syndrome (DS) children, mutations of *GATA1* have been described in all tested cases (Estey *et al*, 2008).

2.1.8.4.1.6 FLT3:

FLT3 is possibly the single most commonly mutated gene in AML. Originally cloned from CD34+ hematopoietic stem cells, it encodes a type III receptor tyrosine kinase in the PDGF receptor family. FLT3 ligand (FL), a type I transmembrane protein expressed on the surface of support and hematopoietic cells in the bone marrow, stimulates growth of immature myeloid cells and stem cells. FL binding induces FLT3 dimerization and activation by cross phosphorylation on intracytoplasmic tyrosine residues. The activated kinase then phosphorylates intermediates in downstream signal transduction pathways. Two types of activating *FLT3* mutations have been identified. The most common are internal tandem repeat duplications (ITD), in which head-to-tail duplications of various lengths and positions occur in the juxtamembrane (JM) portion of the molecule. These may occur due to DNA replication errors as a result of a potential palindromic intermediate that may form at that site. The juxtamembrane domain is an autoinhibitory domain whose function is blocked by autophosphorylation the in frame insertions in the JM domain produce mutant proteins which are constitutively activated by autodimerization and phosphorylation in the absence of ligand. The second type of mutations is activation loop point mutations, usually an Asp825- Tyr substitution resulting from a point mutation. Usually the activation loop inhibits access of ATP and substrate to the kinase domain until phosphorylation occurs as a result of ligand binding. The activation loop mutations again produce a constitutively active FLT3 protein. Interestingly, FLT3 activity on downstream signal transduction intermediates is altered by these mutations. Ligand-activated wild-type FLT3 stimulates proliferation via activation of the Ras/Raf/MAP kinase

pathway. In contrast, FLT3-ITD only weakly activates MAP kinase, but strongly activates STAT5. The frequency of FLT3-ITD in adult AML is 24%, 10– 15% in pediatric AML (reviewed in). FLT3-ITD is detected very frequently in APL, but has been detected in all DN-AML subtypes, and in MDR-AML. The frequency is very low in MDS and ALL. In contrast, the FLT3 activation loop mutation is found in 7% of AML, 3% of MDS, and 3% of ALL. FLT3-ITD is readily detected by PCR of genomic DNA using primer pairs that span the internal tandem repeat region, the amplified abnormal product being easily detected by agarose gel electrophoresis. The expression of FLT3 may be the most significant current prognostic factor for poor outcome in patients less than 60 years old. In a study of 91 pediatric AML patients, event-free survival at 8 years was 7% for patients with FLT3-ITD compared to 44% for patients with wild-type FLT3. In adults with AML from 16 to 60 a Laboratory Studies for Diagnosis and Monitoring of AML 37 years with otherwise normal cytogenetics, presence of FLT3-ITD significantly decreased the length of remission and overall survival. As with BCR-ABL for CML, the contribution of a constitutively active FLT3 tyrosine kinase to the pathogenesis of AML introduces the possibility of selective kinase inhibition as a specific treatment strategy. Small molecule FLT3 kinase inhibitors that bind to the ATP recognition domain of the enzyme have been identified, and several have been tested in phase I clinical trials, with limited frequency and duration of responses. Their use combined with traditional chemotherapy or combined with other classes of inhibitors is being investigated (Estey *et al*, 2008).

2.1.8.4.1.7 Other Tyrosine Kinases and RAS Mutations in AML:

Recurring mutations have been described in AML in other members of the PDGF receptor tyrosine kinase family. Mutation of cKIT occurs in about 50% of CBFmutated (AML1 or CBF) AML cases. In addition, activating RAS mutations have been described in 19% of AML , and activating mutations of cFMS have been described in AML, primarily with M4 or M5 morphology. Interestingly, the PDGF receptor tyrosine kinase family functions to drive proliferation through RAS, and RAS, cKIT, cFMS, and FLT3 mutations appear to be mutually exclusive in AML, suggesting all are contributing the same function in leukytosis (Estey *et al* ,2008).

2.1.8.4.1.8 Nucleophosmin:

Nucleophosmin (NPM) is a molecular chaperone that shuttles between cytoplasm and nucleus, with particular nucleolar concentration of protein. While NPM appears to function to transport preribosomal particles from the nucleolus to cytoplasm, other functions have also been described, including regulation of centrosome duplication, regulation of p53, and functional regulation and stabilization of p19ARF. A possible mechanism by which NPM mutation contributes to leukemogenesis remains undefined. Its frequent association with FLT3 mutation suggests it does not function to drive proliferation of leukemic cells, while in translocations it partners with both ALK1 (another tyrosine kinase, in anaplastic large cell lymphoma) and RAR (Estey *et al*, 2008).

2.1.9 Treatment:

2.1.9.1 Supportive Care:

As for all walking exercise can relieve fatigue. Adequate hydration and allopurinol are essential at the start of treatment to reduce the risk of hyperkalaemia, hyperuricaemia, and renal damage. Psychological and social support to patients and families of patients with leukaemia is important (Drew, 2003)

2.1.9.2 Chemotherapy:

Is very intensive resulting in long period for marrow suppression with neutropenia and thrombocytopenia. The main drugs used include daunorubicin and cytarabine with – 5 cycles given in 1 week blocks to achieve remission (Murray *et al* , 2010).

The cytotoxic effect, improves the chance of remission after the initial “induction” period, and reduces the emergence of drug resistance. In Britain acute myeloid leukaemia is currently treated with four or five courses of intensive chemotherapy when there is intent to cure. Each course entails up to 10 days of chemotherapy. Subsequent courses are commenced after cell counts have recovered and response to treatment is established. During the recovery phase the patient is severely myelosuppressed and needs inpatient blood product support and antimicrobial drugs. In acute myeloid leukaemia M3 the drug ATRA (all-

trans-retinoic acid) is used as an adjunct to chemotherapy as it causes differentiation of the malignant clone, followed by long term maintenance or continuation treatment for up to two years. This has been shown to improve long term cure rates in acute lymphoblastic leukaemia, though not in acute myeloid leukaemia. Some aggressive leukaemia subtypes such as adult T cell leukaemia and Burkitt's lymphoma/leukaemia have shown marked responsiveness to short term intensive treatment schedules. Complete remission rates of 65-86% have been achieved in a disease that previously had an extremely poor prognosis (Drew, 2003).

Treatment directed at the central nervous system the treatment or prevention of leukaemic cells in the central nervous system is part of all treatment protocols in childhood leukemia and adult acute lymphoblastic leukemia, but not in adults with acute myeloid leukemia unless they have symptoms or blasts are present in the cerebrospinal fluid. Treatment directed at the central nervous system generally comprises regular intrathecal chemotherapy (usually methotrexate), high dose intravenous methotrexate, or cranial irradiation (Drew, 2003).

2.1.9.3 Remission Induction Regimens Give the Best Outcomes:

A remission (or complete remission) is usually defined as having no evidence of leukemia after the 4 to 6 weeks of induction treatment this mean:

- the Bone marrow contains fewer than 5%blast cells.
- the blood cells count within normal limit.
- there are no signs or symptoms of the disease

A molecular complete remission means there is no evidence of leukemia cells in the bone marrow, even when using very sensitive lab tests such as PCR .Even when leukemia is in remission, this doesn't always mean it has been cured (American Cancer Society ,2015) .

The most common remission induction regimen used in patients with AML is cytarabine given by continuous intravenous infusion daily for seven days plus an anthracycline such as daunorubicin given daily for three days (the "7+3" regimen). Depending on age and patient selection, 50% to 80% of patients achieve CR. By the 1980s, Preisler et al. had demonstrated an overall CR rate of 66% using the 7+3 regimen; patients less than 60 years old without

a history of prior malignancy had an 80% CR rate. The Cancer and Leukemia Group B (CALGB) demonstrated that a 7+3 regimen with infusional cytarabine was superior to bolus cytarabine and to other combination schedules such as “5+2”. Studies that altered the 7+3 regimen by extending the cytarabine schedule to “10+3” or by adding 6-thioguanine to 7+3 (TAD or DAT) did not significantly improve CR rates. Thus, these early trials all contributed to the standardization of the 7+3 induction regimen (Mark *et al*, 2008).

2.1.9.4 Duration Treatment Approach:

t(8;21)(q22;q22) [AML1/ETO] High Long Standard induction with cytarabine and an anthracycline; intensive consolidation with several courses of high-dose cytarabine inv(16)(p13;q22) or t(16;16)(p13;q22) [CBF β /MYH11] High Long Standard induction with cytarabine and an anthracycline; intensive consolidation with several courses of high-dose cytarabine t(15;17)(q22;q11–12) [PML/RAR α] High Long All-trans-retinoic acid together with an anthracycline for induction; arsenic trioxide for consolidation and to treat relapse t(9;11)(p22;q23) [AF9/MLL] High Intermediate Standard induction and intensive consolidation with high-dose cytarabine; reserve stem cell transplantation for second remission for most t(9;11) patients. Normal karyotype with NPM1 mutation High Long Standard induction and consolidation. No advantage to allo HCT in CR1 del(5q), +13, +8, -7, inv 3, del(12p), t(9;22), other t(11q23), or complex abnormalities Low Short New induction regimens, including use of growth factors during or after chemotherapy, or modulators of drug resistance; perform stem cell transplantation in first complete remission. Mitoxantrone is a synthetic anthracycline analogue (anthracenedione) that has also been compared with daunorubicin for its effectiveness in induction therapy together with cytarabine. Randomized trials have compared these two agents in both younger and older adult populations. Although early trials showed differences in CR rates, OS, and remission duration favoring mitoxantrone, the differences were not statistically significant, and later trials failed to show any benefit of mitoxantrone over daunorubicin for induction therapy. Mitoxantrone may have less cumulative cardiotoxicity than daunorubicin but cannot be strongly recommended over daunorubicin for induction therapy (Mark *et al*, 2008).

2.1.9.5 Bone Marrow Transplant :(BMT)

Pluripotent haematopoietic , stem cells are collected from the bone marrow .Allogeneic transplants from HLA –matched siblings or from matched unrelated donors is indicated during first remission in disease with poor prognosis the idea is to destroy leukemic cells and immune system by cyclophosphamide and total body irradiation and then repopulated the marrow by transplantation from amached donor infused IV .BMT allows the most intensive chemotherapy regimen because marrow suppression is not an issue (Murray *et al* , 2010).

2.1.10 Prognosis:

People with acute myelogenous leukemia (AML) may have questions about their prognosis and survival. Prognosis and survival depend on many factors. Only a doctor familiar with a person’s medical history, type of cancer, stage, characteristics of the cancer, treatments chosen and response to treatment can put all of this information together with survival statistics to arrive at a prognosis (Bain,2004).

A prognosis is the doctor’s best estimate of how cancer will affect a person and how it will respond to treatment. A prognostic factor is an aspect of the cancer or a characteristic of the person that the doctor will consider when making a prognosis. A predictive factor influences how a cancer will respond to a certain treatment. Prognostic and predictive factors are often discussed together and they both play a part in deciding on a treatment plan and a prognosis (Bain, 2004).

The Following are Prognostic and Predictive Factors for AML:

2.1.10.1Chromosome Changes:

Many chromosome changes, or abnormalities, are linked with AML and some of them are used as prognostic factors. Good, or favourable, risk means that the person with AML has the following chromosome abnormalities:translocation (part of a chromosome is transferred to another chromosome) between chromosomes 8 and 21,inversion of chromosome 16 and translocation between chromosomes 15 and 17 (Mark *etal*,2008).

2.1.10.2 Poor-Risk Chromosome Changes:

Poor, or less-favorable, risk means that the person with AML has the following chromosome changes: deletion of part of chromosome 5 or 7, translocation between chromosomes 9 and 11, translocation or inversion of chromosome 3, translocation between chromosome 6 and 9, translocation between chromosome 9 and 22, abnormalities of chromosome 11 and complex changes involving several chromosomes (Mark *et al*, 2008)

2.1.10.3 Intermediate-Risk Chromosome Changes:

Doctors consider people with normal chromosomes or chromosome changes that do not fall into the good- or poor-risk categories as intermediate risk. They have a prognosis that is in between good and poor (Mark *et al*, 2008).

2.1.10.4 Gene Mutations

Some people with AML have certain gene mutations. Doctors use these gene mutations as prognostic factors for people with AML.

About 30% of people with AML have a mutation (internal tandem duplication) in the FMS-like tyrosine kinase 3 (FLT3) genes. This FLT3 gene mutation is linked with a less favorable prognosis. Up to 50% of people with AML have a mutation in the nucleophosmin (NPM1) gene. The NPM1 gene mutation is linked with a more favorable prognosis if there are no other abnormalities. Changes to the CEBPA gene are linked to a more favorable prognosis. Overexpression of the ERG gene in people with AML points to a less favorable prognosis (Estey *et al*, 2008).

2.1.10.5 Age:

Younger adults, usually those younger than 60 years of age, have a more favorable prognosis than older adults. This may be because chromosomal abnormalities can happen as a person gets older. Older people may also have other health conditions that make it difficult for them to cope with the side effects of treatments for AML (Estey *et al*, 2008).

2.1.10.6 White Blood Cell Count:

A white blood cell (WBC) count of more than 100,000 at the time of diagnosis is linked with a less favorable prognosis (Mark *etal*,2008).

2.1.10.7 Response to Chemotherapy:

People who reach complete remission after induction chemotherapy have a more favorable prognosis than those who have refractory disease that does not respond to treatment (Mark *etal*,2008).

The response to chemotherapy is measured as the time it takes to reach a complete remission, or complete response. When a complete remission is reached within 4 weeks of starting chemotherapy, the prognosis is more favorable. The prognosis is less favorable when it takes longer to reach complete remission. The prognosis is poorer in people who don't reach a complete remission after chemotherapy (Mark *etal*,2008).

Minimal residual disease (MRD) means that there are leukemia cells, or blasts, in the bone marrow, but they can only be seen using very sensitive tests, such as flow cytometry or polymerase chain reaction (PCR). The cancer cells can't be seen with standard tests, such as looking at the cells under a microscope. In general, people with AML who have MRD any time after the start of consolidation therapy (the continued treatment given to keep leukemia from coming back) have a higher risk of relapse and a poorer prognosis (Mark *etal*,2008).

2.1.10.8 Early Relapses:

An early relapse means that the leukemia returns soon after treatment. It is linked with a less favorable prognosis (Drew,2003).

2.1.10.9 Previous Blood Disorders:

People who already had a blood disorder, such as a myelodysplastic syndrome (MDS), usually have a less favorable prognosis (Drew, 2003).

2.1.10.10 Previous Treatment for Cancer:

AML that develops after treatment for another cancer usually has a less favorable prognosis (Drew, 2003).

2.1.10.11 Infection:

A serious, uncontrolled infection at the time of diagnosis is a less favorable prognostic factor (Drew,2003).

2.10.12 Leukemia Cells in the Central Nervous System

Spread of AML to the brain and spinal cord (called the central nervous system, or CNS) is a poor prognostic factor (Drew, 2003).

2.2 KIT D 816:

KIT encodes the protein KIT, a member of the type III transmembrane receptor protein–tyrosine kinase family .On binding of the ligand stem cell factor, dimerization of 2 KIT proteins occurs, leading to phosphorylation and a signaling cascade promoting cell growth and proliferation. Activating pathogenic variations of *KIT* occur in the juxtamembrane domain and the kinase domain, such as D816V, which stabilizes the activated conformation of the protein. The D816V pathogenic variation causes ligand–independent constitutive phosphorylation and activation of KIT, leading to uncontrolled growth. Because of its transforming ability, D816V may play a major role in SM. The major criterion is the visualization of multifocal dense aggregates (15 mast cells) in a trephine section (confirmed using mast cell tryptase). The minor criteria are (a) 25% of the mast cells are immature or spindle shaped in the trephine section, (b) a *KIT* point mutation at codon 816, (c) presence of extra–cutaneous infiltrate of mast cells that coexpress CD117 with CD2 and/or CD25, and (d) total serum tryptase 20 g/L. Juxtamembrane pathogenic variations and SM patients without the D816V pathogenic variation are sensitive to the tyrosine kinase inhibitor imatinib mesylate (imatinib), but the D816V pathogenic variation confers resistance to imatinib both in vitro and in vivo. Thus detection of D816V in SM patients would indicate that an alternative treatment to imatinib should be sought. Crystal structure analysis of KIT has elucidated a mechanism for imatinib resistance. Imatinib can bind to the kinase domain only in the inactive state. The D816V variation stabilizes the active kinase state, so imatinib is unable to bind and inhibit KIT. Although D816V is common in SM patients, the reported incidence has been highly inconsistent, possibly due to patient heterogeneity or, more

importantly, due to the tissues tested and the detection methods used. Peripheral blood has been used in some SM studies because D816V is thought to originate in a pluripotent hematopoietic progenitor cell and may be present in cell lineages other than mast cells. The number of mast cells present in peripheral blood and the presence of the pathogenic variation in other mature myeloid cell lineages, however, appear to depend on the severity of the disease. Thus bone marrow samples, where the affected progenitor cells and mast cells are most likely to reside, may yield more useful information than peripheral blood, but the mast cell population of bone marrow aggregates can also be low. Accurate detection of variations present in a small fraction of cells can be difficult. A method with high sensitivity is required in which the rare variant allele will not be overwhelmed by the large proportion of wild-type alleles. Numerous techniques have been developed to enhance the detection of the rare allele signal (Stakahashi, 2011).

2.2.1 Effect of KIT D816 Mutation in AML:

Mutations in codon D816 of the *KIT* gene represent a recurrent genetic alteration in acute myeloid leukemia (AML). To clarify the biologic implication of activation loop mutations of the *KIT* gene, 1940 randomly selected AML patients were analyzed. In total, 33 (1.7%) of 1940 patients were positive for D816 mutations. Of these 33 patients, 8 (24.2%) had a t(8;21), which was significantly higher compared with the subgroup without D816 mutations. Analyses of genetic subgroups showed that *KIT*-D816 mutations were associated with t(8;21)/*AML1-ETO* and other rare *AML1* translocations. In contrast, other activating mutations like *FLT3* and *NRAS* mutations were very rarely detected in *AML1*-rearranged leukemia. *KIT* mutations had an independent negative impact on overall (median 304 vs 1836 days; $P = .006$) and event-free survival (median 244 vs 744 days; $P = .003$) in patients with t(8;21) but not in patients with a normal karyotype. The *KIT*-D816V receptor expressed in Ba/F3 cells was resistant to growth inhibition by the selective PTK inhibitors imatinib and SU5614 but fully sensitive to PKC412. Our findings clearly indicate that activating mutations of receptor tyrosine kinases are associated with distinct genetic subtypes in AML. The *KIT*-D816 mutations confer a poor prognosis to *AML1-ETO*-positive AML and should therefore be included in the diagnostic workup. Patients

with *KIT*-D816-positive/*AML1-ETO*-positive AML might benefit from early intensification of treatment or combination of conventional chemotherapy with *KIT* PTK inhibitors (Stakahashi , 2011).

KIT receptor tyrosine kinase mutations are implicated as a prognostic factor in adults with core binding factor (CBF) acute myeloid leukemia (AML). However, their prevalence and prognostic significance in pediatric CBF AML is not well established. We performed *KIT* mutational analysis (exon 8 and exon 17) on diagnostic specimens from 203 pediatric patients with CBF AML enrolled on 4 pediatric AML protocols. *KIT* mutations were detected in 38 (19%) of 203 (95% CI, 14%-25%) patient samples of which 20 (52.5%) of 38 (95% CI, 36%-69%) involved exon 8, 17 (45%) of 38 (95% CI, 29%-62%) involved exon 17, and 1 (2.5%; 95% CI, 0%-14%) involved both locations. Patients with *KIT* mutations had a 5-year event-free survival of 55% (\pm 17%) compared with 59% (\pm 9%) for patients with wild-type *KIT* (P = .86). Rates of complete remission, overall survival, disease-free survival, or relapse were not significantly different for patients with or without *KIT* mutations. Location of the *KIT* mutation and analysis by cytogenetic subtype [t(8;21) vs inv(16)] also lacked prognostic significance. Our study shows that *KIT* mutations lack prognostic significance in a large series of pediatric patients with CBF AML. This finding, which differs from adult series and a previously published pediatric study, may reflect variations in therapeutic approaches and/or biologic heterogeneity within CBF AML (Pollard *et al*, 2005).

CHAPTER THREE

Materials and Methods

3.1 Study Design:

This study is prospective and longitudinal cohort study conducted from May 2015 to May 2017. All patients received idarubicin plus cytarabine or behenoyl cytosine arabinoside 3+7 induction chemotherapy treatment. Compare case and control group of study population .Aimed to detect KIT D816 mutation and its association with patient's outcome in Sudanese patients with Acute Myeloid Leukaemia under chemotherapy treatment in Khartoum State.

3.2 Study Area Population:

This study was conducted in Gafar Ibn Auf paediatric Hospital, Omdurman Millitary Hospital and Radio Isotope Centre Khartoum. The sample size of 30 venous blood samples was collected from diagnosed AML patients who are classified into two groups.one of them who KIT D 816 positive act as cases group and other who KIT D 816 negative act as control groups.

3.3 Sampling and Sample Method:

Individuals who were diagnosed as Acute Myeloid Leukemia were selected convenience non probability way and data collected using self – administrated pre-coded questionnaire and return to hospital recorders which were specifically designed to obtain information that helped in study.

3.4 Inclusion Criteria:

- Diagnosed de novo Acute Myeloid Leukemia patients.
- Confirmed cases of Acute Myeloid Leukemia patients, were under chemotherapy treatment.

3.5 Exclusion Criteria:

-Any patients who may have other type of malignance that may affect the study line.

-Transformed AML patients from other types of malignancies.

3.6 Data Analysis:

The collected data proceed for analysis using SPSS version 19 computerized program and the data presented in form of tables.

3.7 Laboratory Investigations:

3.7.1 Sample:

4.5 Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with 70% ethanol ,the blood was add to the anticoagulant at ratio of 2.5 to 1.5 of 0.1%EDTA solution (0.1%solution of EDTA can use either the disodium or the dipotassium salt) and gently mix .

The samples were extracted to obtain DNA .then placed into appendrof tubes, capped and frozen at -21C used for PCR.

3.7.2 DNA Extraction (by G-spin Total DNA extraction Kit):

G-Spin Total DNA Extraction mini KIT provide fast and easy methods for purification of total DNA from cultured animal cell ,animal tissue, rodent tail, fixed tissue ,animal hair ,gram negative bacteria ,and blood samples for reliable PCR and Southern blotting .furthermore ,we have tested G-Spin Total DNA extraction mini KIT to get more practical data with a lot numbers of biological samples.(**more details about G-spin kit's protocol show in appendices**)

3.7.3 ACB-PCR:

The ACB-PCR assay is a modified method of allelespecific amplification by used 3 primers designed as normal forward, mutant forward and reverses. The ACB-PCR assay is a modified method of allelespecific amplification by used 3 primers designed as normal forward, mutant forward and reverses. .Both normal forward and variant

forward primers are designed to end exactly at the position of the 2447 AT D816V point variation with the relevant terminal 3 base specific for the normal or variant allele (Fig3.1). Additional mismatched bases are introduced near the 3end, which aids in differentiation. Specificity of the reaction is further enhanced by use of a 3 end label or “blocker” introduced into the 3end of the normal primer, which physically prevents amplification of the normal allele. Under stringent conditions, only the variant allele is amplified (Angia et al, 2006).

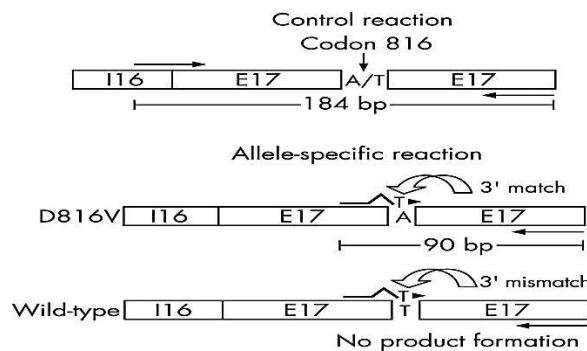


Figure (3.1): Principle of ACB-PCR (Angeia et al, 2006).

3.7.3.5 Basic Elements of Reaction Mixture for PCR:

3.7.3.5.1 Template DNA:

For the many different applications of the PCR, many different methods of isolating and preparing the template DNA exist, mostly depending on the source of the DNA. It is important to note that the outcome of a PCR is dependent on the quality and integrity of the template DNA (Gerril et al,2005).

It is wise to purify template DNA using a product or method that is specifically designed to purify template DNA for use in PCR. The amount of input template DNA is also of crucial importance in PCR and a common mistake is to add too much template DNA to the PCR reaction. Generally, the amount of DNA per reaction should be 10⁴-10⁶ target/template molecules. Blood human template DNA input in 1 ul = 7.5x10⁴ (Gerril et al,2005).

3.7.3.5.2 Primer:(0.1 μ M each 10x.)

Primer obtained from Macrogen Company in aliquot form. the concentrations should be 0.1-0.5 μ M in optimal reactions of a standard or basic nature. As a general rule, 5 pmol of each primer / 25 μ L PCR reaction, 10 pmol/50 μ L PCR reaction and 20 pmol/100 μ L PCR reaction(Gerril et al,2005).

3.7.3.5.2.1 Design of Primers:

The design of primers, more than anything else, will determine the success of a specific PCR assay. The three primers are unrelated to one another, since they anneal to different strands and on opposite ends of the target amplicon. However, special care must be taken to ensure that there is no significant complementarity within or between primers and that primer pairs are balanced with respect to the melting temperature (T_m).

The ACB-PCR assay is a modified method of allelespecific amplification. Three primers, a normal forward, variant forward, and reverse primer combine dintoastandard PCR assay. Both the normal forward and variant forward primers are designed to end exactly at the position of the 2447 AT D816V point variation with the relevant terminal end of the normal primer, which physically prevents amplification of the normal allele. Under stringent conditions, only the variant allele is amplified. The optimal conditions for the ACB-PCR assay were determined by adjusting the annealing temperature, concentration of dNTPs, and primer concentration, all of which affect the specificity of allele-specific PCR reactions. The HMC-1 cell line and normal peripheral blood mononuclear cells were used to determine the optimal conditions for the ACB-PCR assay. If nonoptimal conditions are use dinthe ACB-PCR assay, allele-differentiating primers do not bind specifically, and mispriming will occur from the normal template generating a false-positive product (Gerril et al,2005).

The strands of the duplex can be separated by heat and the temperature at which half the molecules are single-stranded and half are double-stranded, is called the T_m . Thus, the T_m will determine the annealing temperature (T_a) in the cycler temperature profile and should suit all primers. A number of other pointers are also of importance in the

design of primers that will function optimally. All of these criteria can be met in most cases by careful analysis and thought, but for convenience, several primer design software programs are available with most software packages for general DNA sequence analysis in this study used OLIGO 6 software to primer designed which programmed at Alneelin University by Dr.Ibrahim in Molecular lab. See table (3.1).

Table (3.1):primer's sequence design (Angeia et al,2006):

No	Primer Sequence
1	Forward: 5'- GTG ATT TTG GTA TAG CCA GAG A phosphate-3
2	Forward Mutant: 5'- GTG ATT TTG GTC TAG CCA GAA T-3
3	Reverse: 5-AAT CCT TTG CAG GAC TGT CAA G-3

3.7.3.5.2.2 Storage of Primers:

- Oligonucleotides are chemically stable. Left dry, they should be good for years. Once hydrated, they are susceptible to degradation by nucleases. If handled correctly, they should be stable for years
- Any DNA oligonucleotide can be degraded by microbial or fingertip nucleases
- Primers should be stored in deionized water or buffered solutions containing EDTA and kept frozen when not in use.
- Make a concentrated stock solution in water or TE (10 mM Tris pH8.0, 1 mM EDTA).A convenient stock could be 100 μM, stored at -20oC
- We advise that stock solutions be distributed into several tubes for long term storage so that accidental contamination of a tube will not lead to loss of the entire synthesis.

3.7.3.5.2.3 Preparation of Primer Working Solution :

1. Dissolve 50 nmol of primer in 250 μ L DEPC water to make a 100 μ M stock solution.
2. Dilute from this stock 1:9 in DEPEC water to make a working solution at 9 μ M for use in the PCR reactions.
3. Adding 1 μ L of the 10 μ M primer to a 25 μ L PCR reaction will result in a final primer concentration of 0.5 μ M, or 10 pMol of primer in 25 μ L volume.

3.7.3.5.3Maxime PCR PreMix Kit (i-Star Taq):

3.7.3.5.3.1 Description:

INtRON's Maxime PCR PreMix Kit has not only various kinds of Premix Kit according to experience purpose, but also a 2x Master Mix solution. Hot start PCR technique was developed as a method to minimize the deleterious effects of mispriming at lower temperatures during PCR .In a PCR reaction, even short incubation at temperature below the optimum annealing temperature for a particular set of primers can result in mispriming elongation and the subsequent formation of spurious bands.

Maxime PCR PreMix Kit (I-Star Taq)is the product what is mixed every component :i-Star Taq DNA polymerase , dNTP mixure ,reaction buffer and so on-in one tube for 1 rxn PCR .This is the product that can get the best result with the most convenience system .The first reason is that it has every components for PCR, so we can do PCR just add a template DNA ,primer set and D.W.The second reason it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment .In addition, each batches are checked by thorough Q.C. so its reappearance is high .It is suitable for various sample's experience by fast and simple using method.(see Appendices)

3.7.3.5.3.2 Protocol:

1. Add template DNA and primers into Maxime PCR PreMix tubes. Recommended volume of DNA template and primer : 3ul .And appropriate amounts of primers 1 pmol/ul from each primer.

2. Add distilled water into the tubes to a total volume of 18 ul or 50u. Do not calculate the dried components.
3. Dissolve the blue pellet by pipetting.
4. (Option) Add mineral oil.
5. Perform PCR of Samples. Approximately 10–100 ng of DNA was amplified in a total volume of 25 L containing 200 nmol/L of primer Normal_f, 400 nmol/L each of Variant_f and ACB_r, 2.0 mmol/L MgCl₂, 50 mol/L each dNTPs, 0.5 units HotStar Taq and 1x Buffer. After an initial DNA polymerase activation step (95 °C for 30 sec), 35 cycles of 94 °C, 30 s/62 °C, 30 s/72 °C, 45 s were performed with a final 72 °C extension for 5 min. The 99-bp products were visualized on an agarose gel (Adjusted in Alneelin university-Molecular lab).
6. Load samples on agarose gel without adding a loading –dye buffer and perform electrophoresis

3.7.3.5.3.3 Gel electrophoresis:

3.7.3.5.3.3.1 Preparation of 1.5% Agarose Gel:

36ml of distilled water and 4 ml of 10x Tris Boric EDTA were added to 1 gram agarose. The mixture was melted and allowed to cool, 1ul Ethidium Bromide (10mg/ml) were added to the gel and mixed, the mixture was poured into horizontal electrophoresis mini gel tank with a suitable size combs and the gel was left for 20 minutes to polymerize. 4ul of PCR product were mixed with 3ul loading buffer and loaded into the gel. 1ul of DNA molecular weight marker was loaded also. Running buffer was added containing 250 ml distilled water and 1.5 ml 1x TBE buffer. The run was performed at 80-94 volt and a current range 20-30 mA for 40 minutes.

3.7.3.5.3.3.1.1 Precautions to use Ethidium Bromide:

Safety Precautions – EtBr is a potent mutagen and is moderately toxic after an acute exposure. EtBr can be absorbed through skin, therefore, it is important to avoid direct or indirect contact with EtBr. It is an irritant to the eyes, skin, mucous membranes, and upper respiratory tract. Some specific work practices which should be used are as follows:

- When handling EtBr always wear a lab coat, nitrile gloves, and chemical splash.

- Prevent inhalation exposure while working with EtBr powder or crystals by working in a fume hood, or use premixed EtBr solutions or tablets to avoid handling the powder directly.
- Review appropriate EtBr Material Safety Data Sheets (MSDS) before handling EtBr materials.
- Wear UV blocking eye protection when using ultraviolet light to visualize EtBr.
- Wash hands thoroughly after handling EtBr, even though gloves were used (UTHSCSA, 2009).

3.7.3.5.3.3.1.1.1 Spills and Decontamination:

- Call EH&S for assistance for a large spill or any spill in which you are unsure of your abilities to properly handle.
- Use soap and water mixtures or 70% ethanol to wipe clean laboratory work surfaces contaminated with EtBr (UTHSCSA, 2009).

3.7.3.5.3.3.1.1.2 Disposal Procedures for Gel contain Ethidium Bromide:

Allow gels to dry out as much as possible and then place in a clear, labeled bag. Dried gels may be placed in a bag with EtBr contaminated debris(gloves,wipes,etc.) (UTHSCSA,2009)

Submit hazardous waste pick up request to EH&S.

3.7.3.5.3.3.2 Visualization of the Gel :

The gel was visualized on ultra violet trans illuminator and photographed using UV camera with 667 polaroid film .The PCR fragments sizes were estimated from their distance of migration relative to the marker size. We use marker size 100 bp. (length of ampilcon is 99bp to give positive result).

3.7.3.5.3.3.3 Interpretation of Gel Electrophoresis Result:

Positive result:

It Gives ampilcon size 99bp compared with ladder (marker size 100 bp)

3.7.4 CBC (Automated Sysmex technique):

3.7.4.1 Principle of Sysmex:

The coulter principle is based on the following:

Particles suspended in an isotonic diluents, when drawn through an aperture which has an electric current flowing through it will cause a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume. This is also called electrical impedance. (Mark, 2016)

3.7.4.2 Methods of Sysmex:

Whole Blood Mode:

Blood is aspirated from the sample probe into the sample rotor valve:

1-4.0 μl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 μl of diluents and brought to the mixing chamber as diluted sample (1st step dilution).

2-Out of the 1:500 dilution sample 40 μl is measured by the sample rotor valve, diluted into 1:25000 with 1.960 μl of diluent then transferred to the RBCs/plt transducer chamber (2nd step dilution).

250 μl of the sample in the RBCs/plt transducer chamber is aspirated through the aperture. At this time RBCs and plt are counted by the DC detection method. At the same time, hematocrit (Hct) value is calculated by RBCs pulse height detection method. (Mark, 2016)

3.7.4.3 Normal Value of Cell Blood Count:

See table (3.2)

3.7.4.4 Interpretation of a Full Blood Count:

A full blood count must be interpreted with reference to the clinical picture and other pathology results. Caution must be given to certain abnormal results such as low platelets that may be artefact and repeats should be considered. The laboratory will usually investigate abnormalities that may be haematological in nature by assessing the

blood film (by microscopy) and referring this to haematology clinicians where appropriate. In the event of serious abnormalities that require urgent attention, such as; acute leukaemia or severe haemolysis, the laboratory staff will alert the On-Call haematology doctors.

Table3.2: Normal range of cells blood counts of individual's age >6 years:

Parameter	Male	Female
Haemoglobin g/L	135 - 180	115 - 160
WBC x10 ⁹ /L	4.00 - 11.00	4.00 - 11.00
Platelets x10 ⁹ /L	150 - 400	150 - 400
MCV fL	78 - 100	78 - 100
PCV %	0.40 - 0.52	0.37 - 0.47
RBC x10 ¹² /L	4.5 - 6.5	3.8 - 5.8
MCH pg	27.0 - 32.0	27.0 - 32.0
MCHC g/L	310 - 370	310 - 370
RDW	11.5 - 15.0	11.5 - 15.0
Neutrophils	2.0 - 7.5	2.0 - 7.5
Lymphocytes	1.0 - 4.5	1.0 - 4.5
Monocytes	0.2 - 0.8	0.2 - 0.8
Eosinophils	0.04 - 0.40	0.04 - 0.40
Basophils	< 0.1	< 0.1

CHAPTER FOUR

Results

Table (4.1) shows frequency and percentage of case and control :

Sample	Frequency	Percent
Case	23	76.6
Control	7	23.3
Total	30	100

Table (4.2) show frequency and percentage of male and female :

Sex	Frequency	Percent
Male	14	46.7
Female	16	53.3
Total	30	100

Table (4.3) Shows frequency and percentage of subtypes of AML among cases and control groups:

Type	Frequency	Percent
M0	2	6.7
M1	3	10.0
M2	7	23.3
M3	13	43.3
M4	2	6.7
M5	2	6.7
M6	1	3.3
Total	30	100.0

Table (4.4) shows frequency and percentage of AML patient's outcome results under chemotherapy treatment:

Valid	Frequency	Percent
Good	15	50.0
Bad	15	50.0
Total	30	100.0

(4.5) frequency and percentage of KIT D 816 mutation within AML patients comparing with healthy individuals:

Table(4.5) shows significant association between frequency of KIT D 816 mutation and occurrence of AML (P.value =0.00).76% of cases positive for KIT D816 mutation and 23% negative for KIT D 816 mutation .

			KITD816mutation		Total
			Positive	negative	
Sample case	Count	23	0	23	
	% of Total	76.7%	.0%	76.7%	
control	Count	0	7	7	
	% of Total	.0%	23.3%	23.3%	
Total	Count	23	7	30	
	% of Total	76.7%	23.3%	100.0%	

Comparison group	Number	KIT D816	
		Positive	Negative
Healthy individuals	15	0 (0%)	15 (100%)

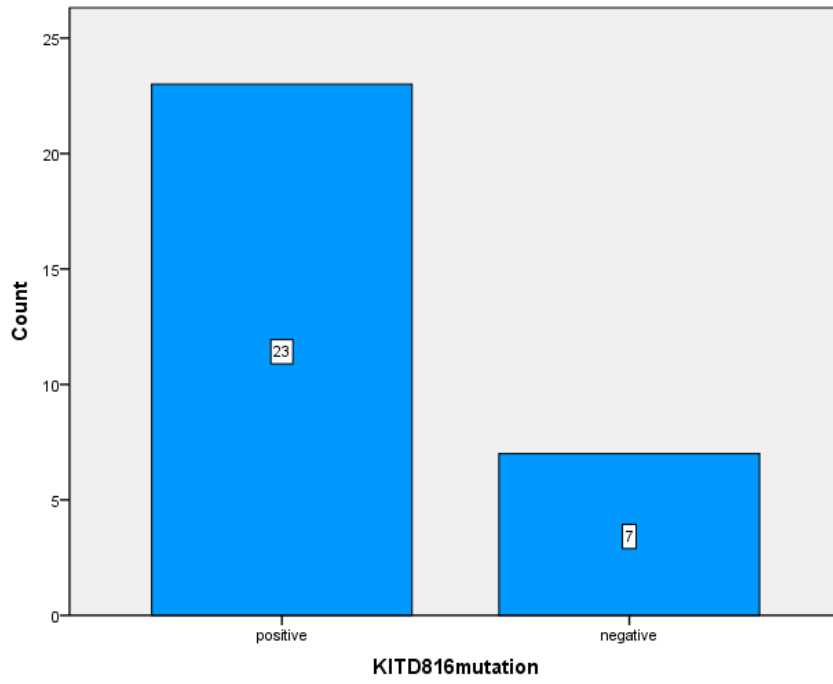


Figure (4.1) Frequency of KIT D 816 mutation among AML patients.

4.6 Comparison of patient's outcome between case group and control group with KIT D 816 mutation for patients under chemotherapy treatments.

Table(4.6) shows insignificant association between KIT D 816 mutation with outcome of patients under chemotherapy treatment(p.value=0.666).

			Outcome		Total
			Good	Bad	
KITD816mutation	Positive	Count % of Total	11 36.0%	12 40.7%	23 76.7%
	Negative	Count % of Total	4 13.3%	3 10.0%	7 23.3%
Total		Count % of Total	16 53.3%	14 46.7%	30 100.0%

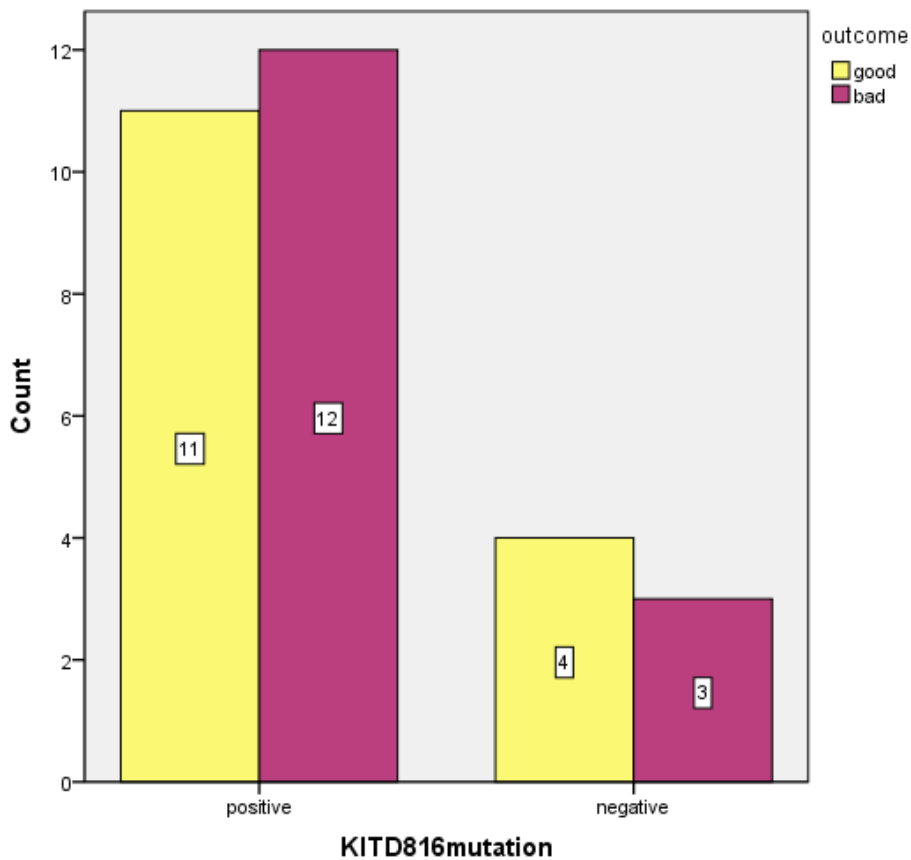


Figure (4.2) Comparison of KIT D 816 mutation's result and patient's outcome.

4.7 comparison of result KIT D 816 mutation among different age of AML patients:

Table(4.7) Shows insignificant association between KIT D816 mutation results and different age of patients among case group (34.83x1000C/cumm±SD) and control group (41.86×1000 C/cumm±SD)(P.value=0.55).

KIT D 816 mutation	N	Mean	P.value
Age positive	23	34.83	0.537
Negative	7	41.86	

4.8 comparison of results KIT D 816 mutation with gender :

Table (4.8) shows significant association between KIT D816 mutations result with gender. (p.value=0.05) by the high incidence of mutation in male more than female.

KITD816mutation		Sex		Total
		Male	Female	
Positive	Count	13	10	23
	% of Total	43.3%	33.3%	76.7%
Negative	Count	1	6	7
	% of Total	3.3%	20.0%	23.3%
Total	Count	14	16	30
	% of Total	46.7%	53.3%	100.0%

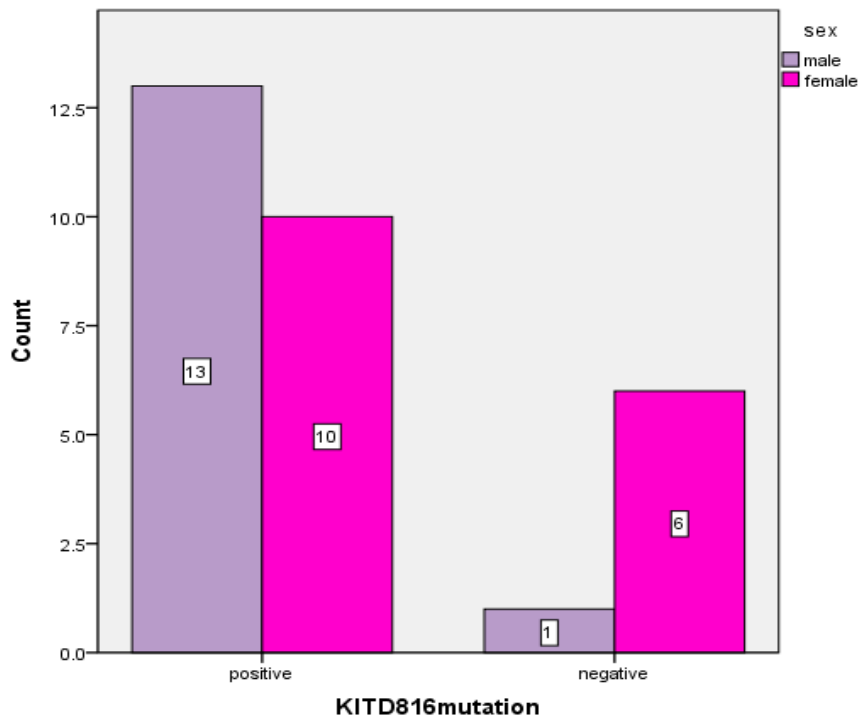


Figure (4.3) comparison of results KIT D 816 mutation with gender

4.9 Comparison of frequency KIT D 816 mutation results combination with different subtypes of AML and patient's outcome

Table (4.9) shows significant association between KIT D816 mutation combination with different subtypes of AML and outcome of patients (p.value=0.008)

		Outcome with KIT D816 mutation				Total
		positive good	negative good	positive poor	negative poor	
subtype AML	M0	Count 0	0	2	0	2
		% of Total .0%	.0%	6.7%	.0%	6.7%
	M1	Count 0	0	1	2	3
		% of Total .0%	.0%	3.3%	6.7%	10.0%
	M2	Count 6	0	0	1	7
		% of Total 20.0%	.0%	.0%	3.3%	23.3%
	M3	Count 6	4	3	0	13
	% of Total 20.0%	13.3%	10.0%	.0%	43.3%	
M4	Count 0	0	2	0	2	
	% of Total .0%	.0%	6.7%	.0%	6.7%	
M5	Count 0	0	2	0	2	
	% of Total .0%	.0%	6.7%	.0%	6.7%	
M6	Count 0	0	1	0	1	
	% of Total .0%	.0%	3.3%	.0%	3.3%	
Total	Count 12	4	11	3	30	
	% of Total 40.0%	13.3%	36.7%	10.0%	100.0%	

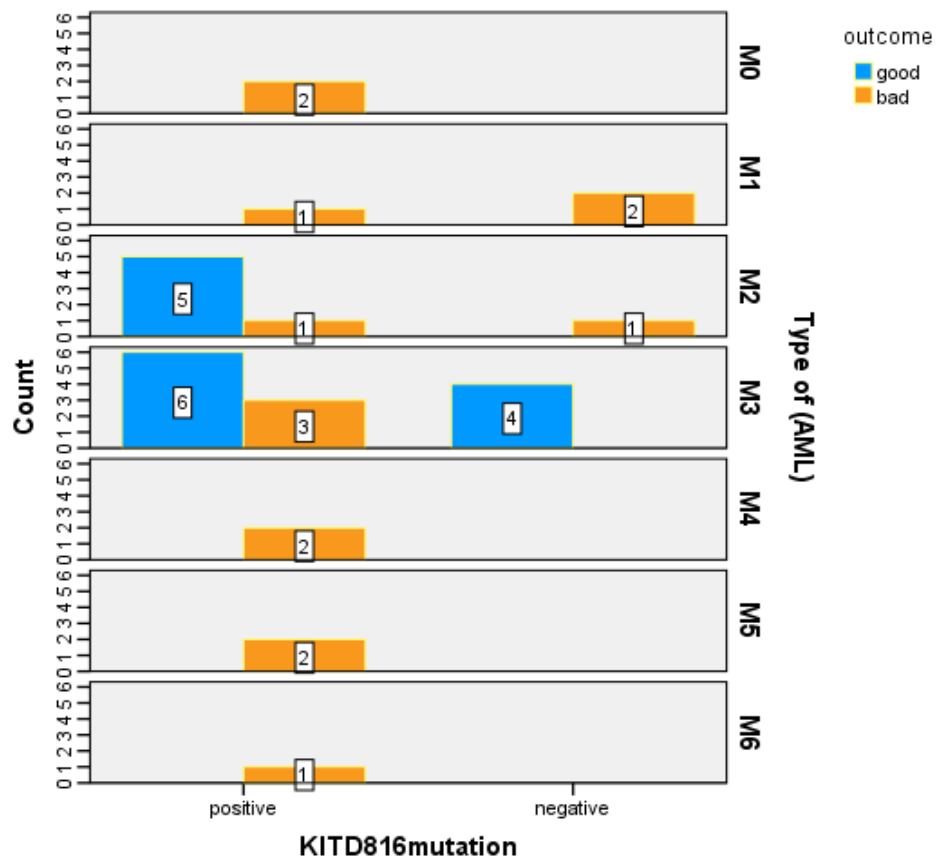


Figure (4.4): comparison of KIT D 816 mutation's results within different subtypes of AML and patient's outcome.

CHAPTER FIVE

Discussion, conclusion and recommendations

5.1 Discussion

This is prospective and longitudinal cohort study was conducted in Radio isotope center, Omdrman Millitary hospital and GafarIbn auf paediatric hospital during the period of May -2015 to May 2017. The study included 30 patients ,14 male and 16 female with Acute Myeloid Leukaemia in different FAB classification of AML subtypes (M0,M1,M2,M3,M4,M5and M6) classified into two groups :case group and control group .Also I run 15 samples from healthy individuals as technical control and to check the mutation among healthy individuals . The age was range from (5-70 years) and duration of disease from 6 months to 2 years.4.5 ml venous blood sample that taken from each patient which divided into two categories: 200ul from these blood was extracted to DNA in the first time collected the samples to analysis KIT D816 mutation and the remaining sample run to did CBC test to follow up the patients.

By ACB-PCR technique this study showed significant association between frequency of KIT D816 mutations and incidence of AML disease (p.value=0.00) the percentage (76.6%) of patients positive for KIT D 816 mutation and (23.3%) of patients negative for KIT D 816 mutation and this result dissimilar to a study (Susanne *et al* ,2005)which has been carried on 1940 AML patients randomly, showed insignificant association between frequency of KIT D816 mutation and incidence of AML.

According to analysis results of KIT D 816 mutation by Allel Specific Competitive Blocker PCR assay classified the patients into two groups: Case group whose were positive for KIT D816 mutation contains 23 patients and control group whose were negative for KIT D 816 mutations that contains 7 patients'. All patients in both groups received idarubicin plus cytarabine or behenoyl cytosine arabinoside 3+7 induction chemotherapy treatment. Then follow up all patients in case and control groups monthly via collected 4.5 ml venous blood to did CBC test and

check clinical findings of all patients in both groups to evaluate patient's outcome. That was minimum period of follow up 5 months.

There was showed insignificant association between KITD816 mutation and AML patient's outcome whose were under chemotherapy treatments as general (p.value=0.666) and this result agree with report (Jesica *et al* ,2009) in pediatric AML patients but disagree to study (Roberto *et al* ,2005) within Italiano AML adult patients .

Also this study showed significant association of KIT D816 mutation when was combined with some types of AML and patient's outcome

(p.value=0.008).in this study KIT D 816 mutation when was present with M0,M4 and M5 can lead to adverse patient's outcome; in spite of (M4) one of favourable outcome factors in AML; in another hand KIT D 816 mutation when was present with M2 and M3 had good outcome by comparing with patient's outcome of control group whose were free from mutation and this result was also in cross ponding with the results of (Schnittger *et al* ,2006) who had defined the reason in KIT D816 variation stabilizes the active kinase state which lead to causes ligand-independent constitutive phosphor- relation and activation of KIT, leading to uncontrolled growth, so imatinib is unable to bind and inhibit KIT therefore patients can't response to chemotherapy treatment and then caused adverse outcome .

Also there is significant association between frequency of KIT D 816 mutations and gender which is affected male patients more than female when comparing with control group that frequency of female patients free from mutation more than frequency of male patients whose were negative KIT D816 mutation (p.value=0.05). And there was insignificant association between frequency of KIT D816 mutation and age (p.value=0.55).

5.2 Conclusion:

-KIT D 816 mutation had highly frequency in AML disease

-KIT D 816 mutation is affected male more than female

-Generally it had insignificant affect in outcome of AML patients under chemotherapy treatment but had significant association with adverse outcome if KIT D 816 mutation presence with some subtypes of FAB classification of our AML patients such as M0,M4 and M5.

5.3 Recommendations:

- More researches in KIT D 816 mutations and association with cytogenetic analysis in Acute leukemic patients.
- Determine the best chemotherapy treatment plan for AML patients associated with KIT D 816 mutation in AML subtypes (M0,M4 and M5).
- KIT D 816 mutation analysis should be included in the AML diagnostic workup.

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**Detection KIT D816 mutation and association with
Sudanese patient's outcome of acute myeloid leukaemia at
Khartoum state.**

Questionnaire

1-NO of sample :.....

2-Age:.....

3-Gender : M..... F.....

4-Duration of disease

5- Type of treatment.....

-Investigations:

-ACB-PCR result:.....

- Case Control

-CBC pre and post treatment :

Pre CBC:

Post CBC :

WBC

WBC

RBC

RBC

HGB

HGB

HCT

HCT

MCV

MCV

MCH

MCH

MCHC

MCHC

PLT

PLT

**After understanding the contents of this questionnaire and the aim of
research I agree To collect the sample.**

Signature:Date.....

وماتوفيقي الا بالله ☺