Chapter one

Introduction and Literature Review

1.1 General introduction:

Neoplastic diseases are in general accompanied by raised levels of ferritin in the plasma. It is suggested that the increase in plasma ferritin may be related to an increased production of ferritin by the malignant cells. In leukemia the normal concentration of ferritin in circulating leukocytes is increased up to six-fold in acute myeloblastic leukemia, more than twenty-fold in acute myelomonocytic leukemia, and two to three folds in chronic granulocytic leukemia. In the presence of various solid tumours, including tumours of the breast, pancreas and liver, an increase of H-subunit rich ferritin was shown in the cells of the tumours, as well as an increase in plasma ferritin. H-subunit rich ferritin can antagonize the cytokine-induced apoptosis by sequestration of iron, preventing reactive oxygen species (ROS) accumulation (Koorts and Viljoen, 2011).

Many factors are suggested to contribute to the hyperferritinaemia associated with cancer, including inflammation, hepatic necrosis due to metastasis and chemotherapy, blood transfusions and a decrease in hepatic clearance of ferritin. In addition, a modified and increased synthesis and secretion of ferritin by tumour cells occur (Koorts and Viljoen, 2011).

1.2 Literature review:

1.2.1 Leukemia:

The leukaemias are group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood (Hoffbrand *et al*., 2006).

1.2.1.1 Classification of leukemia:

The main classification is into four types: acute and chronic leukemias, which are further subdivided into lymphoid and myeloid (Hoffbrand *et al*., 2006).

1.2.1.2 Molecular basis of leukemia:

Genetic predisposition to cancer operates through inherited genetic variants that are either oncogenic themselves or which accelerate the rate of acquisition of somatic oncogenic mutations (Hoffbrand *et al*., 2011).

1.2.1.2.1 Types of somatic mutations:

Cancer is a product of somatic mutations, which can be large-scale (e.g. chromosomal translocations, inversions and numerical aberrations) or small-scale (e.g., point mutations, microdeletions and epigenetic changes) (Hoffbrand *et al*., 2011).

1.2.1.2.1.1 Chromosomal translocations:

Chromosomal translocations are probably the most extensively studied genetic abnormalities in haematological malignancies. Balanced translocations involve a reciprocal exchange of genetic material between two chromosomes and may result in aberrant function of genes adjacent to the breakpoint. Two common mechanisms have been described: First, a fusion gene may be generated encoding a fusion protein with oncogenic properties. This mechanism is seen in many of the translocations associated with myeloid malignancies and some associated with acute lymphoblastic leukemia (ALL). The second category of translocations results in a structurally intact gene being placed next to regulatory elements from a gene on the partner chromosome. This scenario is frequently observed in lymphoid malignancies in which a malfunction in the normal process of

antigen receptor rearrangement results in translocations involving immunoglobulin or T cell receptor (TCR) loci (Hoffbrand *et al*., 2011).

1.2.1.2.1.2 Large deletions and aneuploidy:

Chromosome deletions and disorders of chromosome number (aneuploidy) are frequently seen in haematological malignancies. It is thought that quantitative chromosomal changes of this sort contribute to tumorigenesis by altering the expression levels of key oncogenes or tumour-suppressor genes. However, it is likely that in many cases such chromosomal changes may not be directly pathogenic in themselves, but simply a consequence of genomic instability. Hyperdiploidy is the most frequent cytogenetic abnormality in childhood ALL and can involve any chromosome. Trisomy 8 is the most common numerical abnormality of clonal myeloid disorders and can be seen in acute myeloblastic leukemia (AML), myelodysplasia and myeloproliferative disorders (Hoffbrand *et al*., 2011).

1.2.1.2.1.3 Submicroscopic mutations:

Much of our initial understanding of the molecular pathogenesis of haematological malignancies came from studies of cytogenetically visible chromosomal abnormalities. However, it has since become clear that submicroscopic mutations also play a similarly critical role in many malignancies. Small - scale mutations such as small deletions can disrupt two or more genes. Activating mutations commonly involve oncogenes encoding tyrosine kinases and members of the receptor tyrosine kinases (RAS) pathway and are usually missense mutations or tandem duplications, e.g. around 90% of cases of systemic mastocytosis, particularly those with bone marrow involvement, have an activating point mutation in the v-kit

hardy-zuckerman 4 feline sarcoma viral oncogene (KIT) gene (Hoffbrand *et al*., 2011).

1.2.1.2.1.4 Epigenetic effects:

In a normal cell, epigenetic mechanisms control several important functions including gene transcription, deoxyribonucleic acid (DNA) replication, imprinting and X-inactivation. Somatically acquired epigenetic changes affecting these processes can alter gene expression and thus have a role in tumorigenesis just as genetic mutations can. Methylation of DNA sequences within regions involved in the regulation of gene expression is generally associated with transcriptional silencing of the associated genes. Disordered transpatterns of DNA methylation have been found in a wide variety of haematological malignancies, e.g. there is global genomic hypomethylation in chronic lymphocytic leukemia (CLL) lymphocytes and AML blasts compared with normal haemopoietic cells (Hoffbrand *et al*., 2011).

1.2.2 Acute myelogenous leukemia:

AML is a heterogeneous group of leukemias that arise in precursors of myeloid, erythroid, megakaryocytic, and monocytic cell lineages. These leukemias result from clonal transformation of hematopoietic precursors through the acquisition of chromosomal rearrangements and multiple gene mutations (Rubnitz *et al.*, 2010).

1.2.2.1 Incidence and epidemiology:

Incidence rate of leukemia worldwide is 2.5% and mortality rate is 3.2%. For African the incidence rate is 2.8% and mortality is 3.6%. While, in Sudan leukemic rate is 5.4% and mortality rate is 6.5% with increase the incidence among males which is 7.5% and mortality of 8.4% than in females

of 3.5% and mortality of 4.5% according to International Agency for Research on Cancer (IARC) cancer database (Ferlay *et al*., 2012).

AML can occur in patients of any age, but in general, both the overall incidence and the proportion of total acute leukaemias that are myeloid increase with age. Thus, ALL predominates in children, with only one case of AML diagnosed for every five cases of ALL. For childhood AML, peak incidence occurs in the first year of life, and then decreases until age 4, and thereafter remains relatively constant until adulthood. The incidence of AML then increases through adulthood, during which period 70%- 80% of acute leukemia are AML, with a marked spike in incidence in the elderly. Much of this increased incidence is attributable to AML with myelodysplasia-related changes, which becomes more common with age, while the incidence of de novo AML remains approximately constant across all adult age groups (Foucar *et al*., 2010).

1.2.2.2 Pathophysiology:

AML is a malignant clonal disorder of immature cells in the haemopoietic hierarchical system. Leukemic transformation is assumed to occur in many cases at the level of the haemopoietic stem cell before it has embarked on any lineage commitment. Some cases may originate at a slightly later stage in cells that are committed to lineage differentiation. These cells have abnormal function characterized by failure to progress through the expected differentiation programme and/or to die by the process of apoptosis. Associated with this may be retention of the stem cell characteristic of self-renewal. This leads to the accumulation of a clone of cells, which dominates bone marrow activity and leads to marrow failure (Hoffbrand *et al*., 2011).

1.2.2.3 Risk factors of AML:

The development of AML has been associated with several risk factors, as summarize in (Table 1.2), generally, known risk factors account for only a small number of observed cases. These include age, antecedent hematologic disease, and genetic disorders as well as exposures to viruses, radiation, chemical or other occupational hazards and previous chemotherapy. Most cases of AML arise de novo without objectifiable leukemogenic exposure (Deschler and Lübbert, 2006).

Table 1.1 Selective risk factors associated with AML (Deschler and Lübbert, 2006).

Risk factors	e.g.
Genetic disorders	Down syndrome, Klinefelter syndrome, Patau syndrome, Fanconi anemia, Ataxia-telangiectasia, Schwachman syndrome, Kostmann syndrome, Neurofibromatosis, Li-Fraumeni syndrome.
Physical and chemical exposures	Benzene, drugs such as Pipobroman, Pesticides, cigarette smoking, embalming fluids, Herbicides.
Radiation exposure	Nontherapeutic and therapeutic raddiation.
Chemotherapy	Alkylating agents, Topoisomerase II inhibitors, Anthracyclines, Taxanes.

1.2.2.4 Classification of AML:

The World Health Organization (WHO) classification of AML incorporates and interrelates morphology, cytogenetics, molecular genetics, and immunologic markers in an attempt to construct a classification that is

universally applicable and prognostically valid (Brunning *et al.*, 2001). The most significant difference between the WHO classification and French-American-British (FAB) classification shown in (Table 1.3) is the WHO recommendation that the requisite blast percentage for the diagnosis of AML be at least 20% blasts in the blood or bone marrow. The FAB scheme required the blast percentage in the blood or bone marrow to be at least 30% (Jaffe *et al.*, 2001).

Table 1.2 Classification of AML according to FAB groups (Hoffbrand *et al.*, 2006).

M0	AML undifferentiated.
M1	AML without maturation.
M2	AML with granulocytic maturation.
M3	Acute promyelocytic.
M4	AML with granulocytic and monocytic maturation.
M5	AML with monoblastic (M_{5a}) or monocytic maturation (M_{5b}).
M6	Erythroleukemia.
M7	Megakaryoblastic leukemia.

1.2.2.5 WHO classification:

There are two categories of WHO classification of AML which are:

1.2.2.5.1 Biological subtypes of AML:

There are essentially two ways to categorize specific biologic subtypes of AML:

❖ Molecular Genetic Biologic subtypes:

- AML with t(8; 21) (q22; q22); RUNX1-RUNX1T1.
- AML with inv(16) (p13.1;q22) t(16;16)(p13.1;q22); CBFB-MYH11.
- AML with t(15; 17) (q22; q21); PML-RARA.

- AML with t(9; 11) (p22; q23); MLLT3-MLL.
- AML with t(6; 9) (p23; q34); DEK-NUP214.
- AML with inv(3) (q21; q26.2) t(3; 3) (q21; q26.2); RPN1-EVI1.
- AML with t(1; 22) (p13; q13); RBM15-MKL1.
- AML with mutated NPM1.
- AML with Mutations of CMBPA (Foucar *et al.*, 2010).

❖ Clinical biologic subtypes:

- AML with Characteristic Genetic Abnormalities.
- AML with Multilineage Dysplasia.
- AML and MDS Therapy Related: Secondary to Alkylating agent or Topoisomerase II inhibitor therapy (Foucar *et al.*, 2010).

1.2.2.5.2 AML, not otherwise categorize:

Despite attempts to utilize a biologic-based classification of AML to the greatest extent possible, the 2008 WHO classification has included eleven subtypes of AML, not otherwise categorize (NOS). A lineage-based system is used to subclassify those cases of AML that lack any specific AML-defining biologic characteristic:

- AML with minimal differentiated.
- AML without maturation.
- AML with maturation.
- AMML.
- AMML t(8;16)(p11;p13);MYST3-CREBBP.
- Acute monocytic leukemia.
- Acute erythroid leukemia.
- Acute megakaryoblastic leukemia.
- Acute basophilic leukemia.
- Acute panmyelosis with myelofibrosis (Foucar *et al.*, 2010).

1.2.2.6 Prognostic factors of AML:

The heterogeneity of disease has become apparent with respect to differences in relapse risk. On multivariate analysis a number of factors have emerged that can predict the risk of relapse irrespective of treatment schedules used (Hoffbrand *et al.*, 2011).

1.2.2.6.1 Cytogenetics factors:

Based on several large studies, a cytogenetics risk stratification system has been proposal for AML that categorizes specific karyotypic abnormalities as favorable, intermediate, or unfavorable, as shows in (Table 1.4). In patients of sixty years old or more with de novo AML, the significance of some of cytogenetic risk groups may differ from younger patients. A very complex karyotype, defined as more than five chromosomal abnormalities, is associated with an unfavorable risk in older age group (Foucar *et al.*, 2010).

Table 1.3 Prognostic risk of cytogenetic abnormalities in patients less than sixty years old with de novo AML (Foucar *et al.*, 2010).

Prognostic risk group	Cytogenetic finding
Favorable	t(15;17)(q22;q21) t(8;21)(q22;q22) inv(16)(p13;q22)/t(16;16)(p13;q22)
Intermediate	Normal karyotype t(9;11)(p22;q23) del(7q),del(9q) –Y, +11, +13, +21
Unfavorable	Complex karyotype inv(3)(q21;q26)/t(3;3) t(6;9)(p23;q34) –5, –7

1.2.2.6.2 Molecular Genetics factors:

In addition to the gene mutations (NPM1 and CEBPA) that currently define provisional biologic subtypes of AML, there is an ever-expanding catalogue of additional genetic alterations that occur in significant number in AML of various subtypes. These include mutations (e.g. TET2, MLL, KRAS, NRAS, and WT1) and alterations in gene expression levels (e.g. BAALC, ERG, MN1, EVI1, PRAME, and RHAMM). KIT mutations are particularly associated with a relatively adverse prognosis in the core binding factor AMLs [t(8;21) and inv(16)/t(16;16)]. In general, these alterations contribute to leukemogenesis and carry prognostic significance, but in contrast to NPM1 and CEBPA mutations, they do not presently define distinct biologic or clinical entities (Foucar *et al.*, 2010).

Activating FLT3 mutations occur in two forms, only one of which is at present incontrovertibly significant for AML prognosis. So-called Internal tandem duplications (ITD) affecting the juxtamembrane portion of the protein correlate with poor prognosis, an association that has been particularly documented in karyotypically normal cases (Foucar *et al.*, 2010).

1.2.2.6.3 Flow cytometry:

Aside from the fact that certain immunophenotypic profiles tend to "track" with specific AML categories, certain aberrant immunophenotypic finding have prognostic significance. These distinctive immunophenotypic features are also useful in the assessment for minimal residual disease. Potential predictive markers in AML diagnosis are listed in (Table 1.5) (Foucar *et al.*, 2010).

Table 1.4 Potential immunophenotypic markers with prognostic significance in AML at diagnosis (Foucar *et al.*, 2010).

Marker	Prognostic significance
CD25	Adverse overall and relapse-free survival.
CD15	Favorable prognosis.
CD7	Controversial; lower complete remission rate;
CD11b	Adverse prognosis.
CD56	Lower complete remission rate.
LAP	Lower complete remission rate; adverse prognosis.
Expression of lymphoid antigens	Lower complete remission rate (adult). No prognostic significance (pediatric patients).
Early clearance of peripheral blasts	Better disease-free survival.

1.2.2.6.4 Other prognostic factors:

Additional prognostic factors delineated in various AML outcome studies in adults include age, total white blood cells (TWBCs), de novo versus secondary AML, performance status, and rapidity in the clearance of blasts from either blood or bone marrow. Factors predictive of inferior survival include age more than sixteen years old, non-white ethnicity, absence of a related donor, TWBCs$\geq 100\times 10^{9}$/L, and adverse karyotype (Foucar *et al.*, 2010).

1.2.2.7 Clinical manifestation:

Acute presentation usual cause critical ill due to effects of bone marrow failure. Symptoms of anaemia like weakness, lethargy, breathlessness, lightheadedness and palpitations. Symptoms of infection like particularly

chest, mouth, perianal, skin [Staphylococcus, Pseudomonas, herpes simplex virus (HSV), and Candida], Fever, malaise, sweats. Symptoms of hemorrhage [especially AML M3 due to disseminated intravascular coagulation (DIC)] like purpura, menorrhagia and epistaxis, bleeding gums, rectal, retina. In addition to gum hypertrophy and skin infiltration (especially AML M4 and M5). Signs of leucostasis like hypoxia, retinal hemorrhage, confusion or diffuse pulmonary shadowing. Hepatomegaly occurs in 20%, splenomegaly in 24% lymphadenopathy is infrequent 17%. Central Nerves System (CNS) involvement at presentation is rare in adults with AML (Provan *et al*., 2004).

1.2.2.8 Laboratory investigation:

Many laboratory techniques had been improved to apply for investigation and diagnosis of AML. These techniques give accurate specific results which help in management of the disease.

1.2.2.8.1 Counting systems:

Two types of counting systems are known that using for automated blood counters:

1.2.2.8.1.1 Impedance counting:

Impedance counting first described by Coulter, (1956) depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors; this difference forms the basis of the counting system used in Beckman Coulter, Sysmex, Abbott, Horiba Medical and a number of other instruments (Bain *et al*., 2011).

For a cell count, blood is highly diluted in a buffered electrolyte solution. The flow rate of this diluted sample is controlled by a mercury siphon or by displacement of a tightly fitting piston. This results in a measured volume of

the sample passing through an aperture tube of specific dimensions. By means of constant source of electricity, a direct current is maintained between two electrodes, one in the sample beaker or the chamber surrounding the aperture tube and another inside the aperture tube. As a blood cell is carried through the aperture, it displaces some of the conducting fluid and increased the electrical resistance. (Bain *et al*., 2011).

1.2.2.8.1.2 Light scattering:

Red cells and other blood cells may be counted by means of electro-optical detectors. A diluted cell suspension flows through an aperture so the cells pass, in single file, in front of a light sources; light is scattered and detected by a photomultiplier or photodiode, which converts it into electrical impulses that are accumulated and counted. The amount of light scattered is proportional to the surface area and therefore the volume of the cell so that the height of the electrical pulses can be used to estimate the cell volume (Bain *et al*., 2011).

1.2.2.8.2 Blood film:

Blood film can be prepared from fresh blood with no anticoagulant added or from Ethylenediaminetetra acetic acid (EDTA) anticoagulated blood. Good film can be made in the following manner, using clean slides. Slides should measure 75×25 mm and be approximately 1 mm thick, ideally, they should be frosted at one end to facilitate labeling. The film should be about 3 cm in length. It is important that the film of blood finishes at least 1 cm before the end of the slide. Films should be fixed as soon as possible after they have dried. Methyl alcohol (methanol) is the fixation of choice. Romanowsky stains are used universally for routine staining of the blood films and satisfactory result can be obtained. (Bain *et al*., 2011).

1.2.2.8.3 Bone marrow aspiration and trephine biopsy:

Bone marrow aspiration and trephine biopsy are simple and safe procedures that can be repeated many times and can be performed on outpatients. The morphological assessment of aspirated or core biopsy specimens of bone marrow is based on two principles. First, that bone marrow has an organized structure such that in normal health, bone marrow cells display distinct numerical and spatial relationships to each other. Second, that individual bone marrow cells have distinctive cytological appearances that reflect the lineage and stage of maturation. The specimens obtained by bone marrow aspiration or by bone marrow trephine biopsy are very different samples and contribute differently to diagnosis (Bain *et al*., 2011).

1.2.2.8.4 Cytochemistry:

Leucocyte cytochemistry encompasses the techniques used to identify diagnostically useful enzymes or other substances in the cytoplasm of Haemopoietic cells. These techniques are particularly useful for the characterization of immature cells in AML and the identification of maturation abnormalities in the myelodysplastic syndrome (MDS) and myeloprolifrative disorder (MPD) (Bain *et al*., 2011). The most important leucocyte cytochemistry stains are:

1.2.2.8.4.1 Myeloperoxidase:

Myeloperoxidase (MPO) is located in the primary and secondary granules of neutrophils and their precursors, in eosinophil granules and in the azurophilic granules of monocytes. MPO splits H2O2 and in the presence of a chromogenic electron donor forms an insoluble reaction product (Bain *et al*., 2011).

1.2.2.8.4.2 Sudan black B:

Sudan black B (SBB) is a lipophilic dye that binds irreversibly to an undefined granule component in granulocytes, eosinophiles and some monocytes. It cannot be extracted from the stained granules by organic dye solvents and gives comparable information to that of MPO staining (Bain *et al*., 2011).

1.2.2.8.4.3 Neutrophils alkaline phosphatase:

Alkaline phosphatase activity is found predominantly in mature neutrophils, with some activity in metamyelocytes. Early methods of demonstrating alkaline phosphatase relied on the use of glycerophosphate or other phosphomonoesters as the substrate at alkaline PH, with a final black reaction product of lead sulphide (Bain *et al*., 2011)

1.2.2.8.4.4 Acid phosphatase reaction:

Cytochemically demonstrable acid phosphatase is ubiquitous in haemopoietic cells. The staining intensity of different cell types is somewhat variable according to the method used. The reaction product is red with a mixture of granular and diffuse positivity. Granulocytes are strongly positive. Monocytes, eosinophiles and platelets show variable positively (Bain *et al*., 2011).

1.2.2.8.5 Immunophenotyping:

There are several techniques for identifying antigens expressed by leucocytes:

1. Flowcytometry to test suspensions of viable cells or fixed cells.
2. Immunocytochemistry to examine cells on cytospin made slides or directly on blood or bone marrow films.

3. Immunohistochemistry to study cells in frozen or paraffin-embedded sections from bone marrow biopsy specimens or other Haemopoietic tissues (Bain *et al*., 2011).

1.2.2.8.6 Molecular genetic analysis:

Include southern blotting, polymerase chain reaction (PCR) to study genomic DNA and reverse transcriptase polymerase chain reaction (RT-PCR) to study ribonucleic acid (RNA) after its reverse transcription (Hoffbrand *et al*., 2011).

1.2.2.8.7 Cytogenetic analysis:

Classical cytogenetic analysis is carried out on cells that have entered mitosis and have been arrested in metaphase so that individual chromosomes can be recognized by their size and their banding pattern following staining [e.g. Giemsa staining (G-banding) or staining with a fluorescent dye]. Alternating dark and light bands are numbered from the centromere toward the telomere to facilitate description of any abnormalities detected. The result of cytogenetic analysis may be display visually (a karyogram) or written according to standard conventions (a karyotype) (Bain *et al*., 2011).

1.2.2.8.8 Fluorescence *in site* hybridization:

FISH bridges classical cytogenetic analysis and molecular diagnostic techniques. Chromosomes can be stained and visualized but the technique is also dependent on the recognition of specific DNA sequences by means of a fluorescent probe that can anneal to a specific DNA sequence. Fluorescence *in site* hybridization (FISH) can be carried out on metaphase preparations or on cells in interphase (Bain *et al*., 2011).

1.2.2.8.9 Whole-genome scanning:

There are a number of molecular techniques available for whole-genome scanning. These techniques include comparative genomic hybridization (CGH) and microarray analysis (Hoffbrand *et al*., 2011).

1.2.2.9 Laboratory finding:

Hematological investigations may reveal a normochromic, normocytic anaemia with thrombocytopenia in most cases. The total whole blood cell count may be decreased, normal or increased to 200×10^9/L or more. Blood film examination typically shows a variable numbers of blast cells. The bone marrow is hypercellular with > 20% leukemic blasts. The blast cells are characterized by morphology, immunological test and cytogenetic analysis. Biochemical tests may reveal a raised serum uric acid, serum lactate dehydrogenase or, less commonly, hypercalcaemia. Liver and renal function tests are performed as a baseline before treatment begins. Test for DIC are often positive in patients with the promyelocytic (M3) variant of AML (Hoffbrand *et al*., 2006).

1.2.2.10 Treatment:

This may be conveniently divided into supportive and specific treatments. Supportive treatment includes insertion of a central venous catheter, blood product support, haemostasic support, antiemetic therapy, psychological support, nutritional support and prevention of tumor lysis syndrome. Specific therapy is primarily with the use of intensive chemotherapy. This is usually given in four blocks each of approximately 1 week and the most commonly used drugs include cytosine arabinoside, daunorubicin, idarubicin, mitoxantrone and etoposide. ALL the AML subtypes are treated

similarly except for the promyelocytic (AML M3) variant associated with the t(15;17) translocation (Hoffbrand *et al.*, 2006).

All-trans retinoic acid (ATRA) with chemotherapy is the standard of care for acute promyelocytic leukemia (APL), resulting in cure rates exceeding 80%. Pilot studies of treatment with arsenic trioxide with or without ATRA have shown high efficacy and reduced hematologic toxicity (Lo-Coco F *et al.*, 2013).

Autologous stem cell transplantation reduces the rate of relapse but adds further toxicity to the treatment regime and is of no overall benefit. Allogeneic transplantation is used in patients under sixty-five years old with Human leucocyte antigen (HLA) matching sibling donor with standard or poor risk AML (Hoffbrand *et al*., 2006).

1.2.2.11 Relapse:

Relapse of AML is generally characterized by a marked decline in cell counts in blood, variable numbers of circulating blasts, and variable extent of bone marrow effacement. These relapse AMLs generally exhibit similar morphological, immunophenotypic, and genetic features to the original leukemia, although clonal evolution may occur (Foucar *et al.*, 2010).

1.2.2.12 Remission:

AML in remission is defined as a normal peripheral blood cell count (absolute neutrophil count >1,000/mm3 and platelet count >100,000/mm3) (Cheson, *et al.*, 1990) and normocellular marrow with less than 5% blasts in the marrow and no signs or symptoms of the disease. In addition, no signs or symptoms are evident of central nervous system leukemia or other extramedullary infiltration. Because the vast majority of AML patients meeting these criteria for remission have residual leukemia, modifications to

the definition of complete remission have been suggested, including cytogenetic remission, in which a previously abnormal karyotype reverts to normal, and molecular remission, in which interphase FISH or multiparameter flow cytometry are used to detect minimal residual disease. Immunophenotyping and interphase FISH have greater prognostic significance than the conventional criteria for remission [(Cheson *et al.*, 2003) (Bacher *et al.*, 2006)].

1.2.3 Ferritin:

Iron-free ferritin (apoferritin) is asphere with a diameter of 13 nm. It has a hollow central cavity, 6 nm in diameter, which communicates with the surface by six channels, through which iron and other small molecules can enter or leave. The protein shall is constructed of 24 polypeptide subunits of two immunologically distinct kinds, H (heavy) and L (light). The molecular weights of the H and L subunits are about 21,000 and 19,000, respectively. The expressed gene for H chain probably is located on chromosome 11 and that of the L chain is on chromosome 19 (Greer *et al.*, 2009).

There are functional differences between the two subunits. H chains contain a ferroxidase center not found in L chains and are able to oxidize iron at a more rapid rate. Ferritin rich in H chains reflect this property and acquire iron more rapidly. Ferritin rich in L chains appear more stable and resistant to denaturation. When iron is added in the molecule, it is deposited in the central core. Rather than a smooth sphere, the iron core is partially divided into four lobes by protein indentations (Greer *et al.*, 2009).

1.2.3.1 Serum ferritin concentration:

Determination of serum ferritin concentration is the method of choice for evaluating iron stores. Although it is an indirect measure, it has the

advantage over biopsy procedures of being more quantitative as well as noninvasive and therefore less expensive and more acceptable to patients. Ferritin is chiefly an intracellular iron storage protein, but trace amounts are also secreted into plasma. The serum protein differs from the intracellular protein in that it is glycosylated and contains little or no iron. Presumably, serum ferritin is formed on endoplasmic reticulum, whereas storage ferritin is formed on free ribosomes (Greer *et al*., 2009).

Most investigators report a mean value for normal adult men of about 90 to 95 µg/L with a range of about 20 to 250µg/L. for women; mean values of about 35 µg/L is noted, with a usual range of 10 to 200 µg/L, when iron deficiency was excluded. In infants, values are high at birth but rapidly fall to about 30µg/L, where they remain until puberty. During adolescence, higher adult values are established in young men. After menopause, values in women increase and approach those found in men. Serum ferritin values in men tend to rise steadily with age. These levels show little or no diurnal variation (Greer *et al*., 2009). And positively correlate with hemoglobin concentration (Franchini *et al*., 2007).

The serum ferritin level in patients with anemia of chronic diseases may increase disproportionately relative to the increase in iron stores, probably because ferritin is an acute phase reactant. In some other illnesses, the serum ferritin level increase because of factors other than augmented iron stores. One such disorder is liver disease in which damage to the hepatic cell can cause the release of intracellular ferritin (nonglycosylated and iron-rich). Serum ferritin values may be inappropriately increased in association with various malignancies, especially hematological malignancies. Production and release of ferritin by malignant cells is one possible explanation; liver

involvement is another. Increased values were also noted in patients with hyperthyroidism (Greer *et al*., 2009).

Low values for serum ferritin may be found in patients with hypothyroidism or with vitamin C deficiency, but the values are not low enough to be confused with iron deficiency (Greer *et al*., 2009).

1.2.3.2 Intracellular ferritin:

In various populations of peripheral blood leucocytes, the appearance of stainable iron granules in monocytes and lymphocytes from patients with haemochromatosis was the result of iron overload, and measurement of ferritin in normal leucocytes showed no difference in leucocyte ferritin concentration between males and females. However, within a population of leucocytes, monocytes contain a greater amount of ferritin than lymphocytes and polymorphs. Incubation of white blood cells from normal subjects and patients with acute myeloblastic leukemia with ^{14}C-leucine demonstrated increase ferritin synthesis in the leukemic cells compared to normal leucocytes. Although ferritin synthesis in normal and in patient's leucocytes did not seem to be stimulated by increasing concentration of iron, it was suggested the ferritin protein in the leukemic cells has the characteristics of apoferritin with very low iron content and may be a specific leucocyte ferritin (Pattanapanyasat, 1989).

And recently, Cells with a high content of H-subunit rich ferritin include erythroid cells, heart cells, pancreatic cells, kidney cells, lymphocytes and monocytes whereas the L-subunit rich ferritin are found predominantly in liver and spleen organs associated with long-term iron storage. The H-subunit to L-subunit ratio of a specific type of cell does, however, not remain constant and the proportion of the H-subunit and L-subunits present

in the ferritin shell changes during differentiation and in various pathological states (Koorts and Viljoen, 2011).

1.2.4 Previous studies:

Many previous studies had demonstrated the elevating of serum ferritin concentration with haematological malignancies especially AML disease.

Abd El Maksoud *et al*., (2010) in Egypt assessed the prognostic impact of elevated serum hyulronic acid, ferritin and interleukin-6 in patients with AML, sample were (115) subjects, divided into two groups: Group A, patient group consisted of 80 with AML attending the National Cancer Institute, Cairo University. They were 48 males and 32 females. Group B, 35 healthy volunteers were used for comparison with AML patients. Serum ferritin was measured using a commercially available Sandwich Enzyme Linked Immunoabsorbent Assay (ELISA) kit. This study reported that significantly higher serum level of ferritin in newly diagnosed and relapsed patients with AML compared with both control group and leukemic patients in remission stage (p value<0.001). Serum ferritin level in newly diagnosis patients was found to be (1400.25ng/ml) and in patients with relapse was (1431.45ng/ml). In contrast level of ferritin in serum of patients in remission was (99.34ng/ml) and in control group was (36.69ng/ml). Ferritin in patients with AML was correlated inversely with Hb concentration (r = -0.44), while correlated positively with both TWBCs (r = 0.69) and with the % of blast cells in bone marrow (r = 0.79). The study was suggested that serum ferritin level can be used as prognostic serum markers at diagnosis of adult AML and it could be used as follow up parameters for early detection of relapse.

Ahlawat *et al*., (1994) in India investigated role of serum ferritin in assessment of disease activity in acute and chronic leukemia, serum ferritin was estimated by using double antibody sandwich ELISA in 83 patients of

acute and chronic leukemia at various stages of the disease; 28 patients of them have AML. Ferritin in AML patients was 775 ng/ml which was significant higher than control value of 46.14 ng/ml and shown no decline with remission. Study found no correlation with clinical and laboratory parameters like age, sex, fever, organomegaly, Hb concentration and TWBCs.

Jones *et al*., (1973) investigated ferritinaemia in leukemia and Hodgkin's disease. Sample was thirty-five adults with untreated AML, serum ferritin concentration in AML patients found increased about ten times than normal level. Mean ferritin concentration was (589 ± 66 ng/ml), the highest value of AML patients of (2200 ng/ml) was higher compared with the highest normal value of (186 ng/ml). There was no correlation of serum ferritin with Hb concentration, platelet count, and serum iron and transferrin saturation. In contrast, there was a significant correlation between ferritin level and TWBCs ($r=0.49$, $P<0.005$).

Jacobs, (1984) investigated serum ferritin and malignant tumors, found that increase concentration of serum ferritin was common in patient with malignant diseases and appear to be non-specific response. He viewed that the present evidences does not suggest that serum ferritin assay is useful either for specific diagnosis or monitoring of malignant diseases.

Aulbert and Fromm, (1987) investigated the abnormal production and release of ferritin by immature myeloid cells in leukemia. Sample was 176 adults patients with leukemia, 45 cases of them were AML and 40 cases were AMML. Serum ferritin was measured with respect to various hematological parameters before therapy was started and during the course of the disease. And patients were divided into six subgroups according to FAB classification. Ferritin was demonstrated by mean of a standardized

solid phase enzyme immunoassay. The study was reported extremely high serum ferritin in patients with immature AML (M1) and AML (M2) FAB classification before treatment. The mean ferritin concentration of AML (M1) was 2235 ±870 ng/ ml about 22 fold increased. The mean ferritin concentration of AML (M2) was 1355±532 ng/ml which is about 13 fold increased. However serum ferritin level in AMML and AML (M4) and AML (M5) found to be significant lower of 450±252 ng/ml. There was no correlation between serum ferritin level and bone marrow, storage iron, serum iron concentration and transferrin saturation.

Parry *et al*., (1975) investigated serum ferritin in acute leukemia at presentation and during remission, eleven adults with AML aged between 18_62 years were examined at presentation and ten of them after they had achieved a complete clinical, blood, and bone marrow remission. Serum iron concentration and total binding capacity were measured and serum and white cell ferritin concentrations measured using the immunoradiometric assay. Result was showed increase serum and white cells ferritin concentration in untreated patients. In remission patients have ever higher level, the mean increase being (289 µg/L) about 23% over the initial mean value ($p<0.001$). Mean serum ferritin in patients of AML at presentation was (1278±367µg/L) and that in remission was (1567±331 µg/L), mean leucocytes ferritin concentration at presentation was (89.6±26 fg/cell) and (25.7±10.8 fg/cell) in remission. In contrast, leucocyte ferritin concentration decreased during remission and the mean difference of 64 fg/cell about (71%), the whole group was significant ($p<0.05$). Both serum iron concentration and total binding capacity was in normal range.

Tanaka and Kato, (1983) measured ferritin in the leukemic blasts with a "sandwich" type enzyme immunoassay method. The concentration of

ferritin in serum of 36 patients with AML and ferritin within leukemic blasts of 24 patients of acute leukemia were measured with improved method of enzyme immunoassay for ferritin which uses beta-D-galactosidase from Escherichia coli. They study was reported a good correlation between radioimmunoassay and enzyme immunoassay (r= 0.946). Result was markedly increased of serum ferritin in patients of acute leukemia of (698±447 ng/ml) with highest concentration shown of patients with acute monocytic leukemia of (1156±324 ng/ml). In addition, markedly elevated of ferritin concentration contain within leukemic cells of (64.3±38.7 ng/cells) compared with ferritin in normal leukocyte of (7.1±2.8 ng/cells), blasts from patients with acute monocytic leukemia was showed the highest level of (105±26.6 ng/cells).

Nevertheless, to the best of our knowledge there were no previous studies found in Sudan that demonstrated serum ferritin level in leukemic patients with AML or its significance.

1.2.5 Rational:

Ferritin, a major iron storage protein, is essential to iron homeostasis and involved in a wide range of physiologic and pathologic processes. In clinical medicine, ferritin is predominantly utilized as a serum marker of total body iron stores. In cases of iron deficiency and overload, serum ferritin serves a critical role in both diagnosis and management. Elevated serum and tissue ferritin are linked to malignancy (Knovich *et al*., 2008).

Many previous studies around the world demonstrated the relationship of serum ferritin level and leukemia like Abd El Maksoud *et al*., (2010) and Ahlawat *et al*., (1994). Although leukemia in Sudan is the fourth most common cancer in men and the seventh most common cancer in women (Ferlay *et al*., 2012). To the best of our knowledge there was no previous published study in Sudan that demonstrated the relationship between ferritin and leukemia. This study aimed to estimate and assess serum ferritin level among AML patients in Khartoum State.

1.2.6 Objective:

1.2.6.1 General objective:

To study serum ferritin level among acute myeloblastic leukemia patients in Khartoum State.

1.2.6.2 Specific objective:

1. To measure serum ferritin level of AML patients.
2. To correlate serum ferritin level among AML patients according to total leucocytes count and hemoglobin concentration.
3. To identify serum ferritin level among AML patients regarding to FAB classification subclasses.
4. To identify serum ferritin level of AML patients among age and gender.
5. To verify serum ferritin level among AML patients according to blasts percentage in peripheral blood.

Chapter Two

Materials and Method

2.1 Study design:

It is a descriptive study, conducted in National Center of Radiotherapy and Nuclear Medicine in Khartoum, Sudan, during the period from Oct 2014 until Nov 2014.

2.2 Study population:

40 diagnosed AML patients based on clinical examination, blood and bone marrow examination, molecular analysis and flowcytometry, who attended the National Center of Radiotherapy and Nuclear Medicine in Khartoum.

2.2.1 Inclusion criteria:

Samples were collected from patients accepted to participant in the study and completed the clinical and laboratory diagnosis of AML of both gander and different age groups.

2.2.2 Exclusion criteria:

AML patients have Iron metabolism disorders, Thalasiemia, non hematological neoplasms and renal diseases.

2.3 Sample size:

Total coverage of the patients attended the National Center of Radiotherapy and Nuclear Medicine in Khartoum during the period from Oct 2014 until Nov 2014.

2.4 Sampling Technique:

Probability simple random sample, 2 ml of venous blood was collected from patients by standard procedures in sterile plain vacotainer. Then serum was

separated by leaving it undisturbed at room temperature and allowed to clotting then clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes. Immediately, Serum was transferred into another sterile blood collection tube by using a Pasteur pipette. Then serum ferritin level was measured by automated A15 Biosystem Chemistry Analyzer (Barcelona, Spain). Hemolyzed and lipemic Samples were excluded.

2.5 Data collection:

Data was collected by using pre-coded questionnaire which was designed to obtain certain data about some variables like age, gender, TWBCs, FAB subclasses, Hb concentration and blast % in peripheral blood. And aimed to either including or excluding certain patients in or out the study.

2.6 Methodology:

Serum ferritin level was measured by Biosystem Chemistry Analyzer A15 (Barcelona, Spain).

2.6.1. Principle of method:

Serum ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies, the agglutination of the latex particles was proportional to the ferritin concentration and can be measured by turbidimetry (Bernard and Lauwerys, 1984).

2.6.2 Reagents:

Reagent obtained by Biosystem latex kit for serum ferritin measurement which was:

- Reagent A: 30 ml Contain of glycine buffer170 mmol/L, sodium chloride 100mmol/L, sodium azide 0.95 g/L, pH 8.2.
- Reagent B: 15 ml of Suspension of latex particles coated with anti human ferritin antibodies, sodium azide 0.95 g/L.

- Ferritin standard: 3 ml of human serum with known ferritin concentration. The concentration value is traceable to the Biological Reference Material WHO 94/572 (NIBSC).
- Working reagent: content of reagent B vial was poured into reagent A bottle. Then working reagent was Mixed thoroughly, this reagent stable for 20 days at 2 – 8 C^o. smaller working reagent volumes were prepared by mixing 1 ml of reagent B + 2ml of reagent A.

2.6.3 Assay procedure:

As Biosystem latex kit instructions shown in (Appendices IV), 10 µL was measured from sample and standard then transferred to sterile tubes. For blank, 10 µL of distilled water was used. Then 200 µL of reagent A was added to all tubes. Mixed and incubated for 3 – 5 minutes. After that 100 µL of reagent B was added to all tubes and mixed well. Finally, the absorbance was read by turbidimetry in 540±20 nm wavelength after 10 seconds and after 5 minutes at 37 C^o.

2.6.4 Normal value:

Men: 20 – 250 µg/L.

Women: 20 – 200 µg/L (Burtis *et al*., 2005).

2.7 Ethical consideration:

Aim of the study was explained for all patients included in the study and informed consent was obtained from them as shown in (Appendices II).The confidentiality of the patients was established by coding of the questionnaire and the data list by different code from their files to insured the anonymity of respondents.

2.8 Data analysis:

Statistical analysis was performed by standard program of SPSS version 17, 2008 statistical package. Statistical One-Sample T Test, Independent-Samples T Test and One-Way ANOVA Test were applied to the data. Correlation Coefficient (r) was used to determine the relationships between different quantitative values. For all tests a probability less than 0.05 was considered significant. Data was presented in the form of tables and figures.

Chapter Three

Results

3.1 Results:

Samples were 40 AML patients with age between 16-67 years old, they were 21 male (52.5 %) with mean age of (41.86±3.69) years old and 19 female (47.5%) with mean age of (40.63±3.24) years old, a study was done to estimate and assess serum ferritin level among AML patients in Khartoum State and to detect the effect of some variables like gender, age, TWBCs, Hb concentration, blasts % and FAB classification on serum ferritin level in these patients.

Regarding to gender males were 21 cases (52.5%) while females were 19 cases (47.5%) in this study that shown in (Fig 3.1).

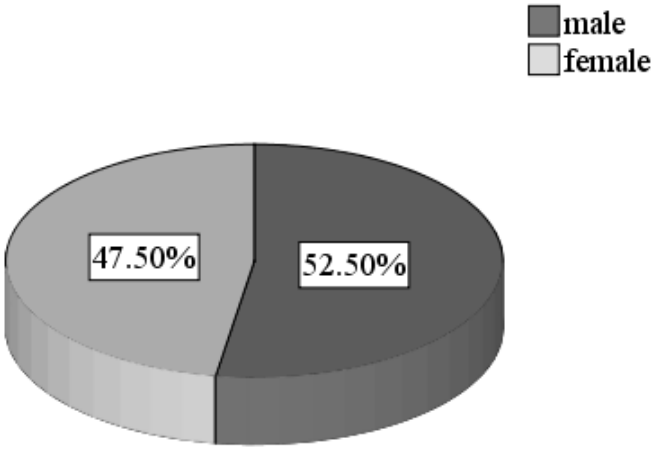

Fig 3.1 Percentage of males and females participate in the study.

(Table 3.1) shows the age of the study population was between 16 – 67 years old and the mean of age among the study groups was (41.27±2.44) years old.

(Table 3.1) Mean age among both genders on study population.

Gender	N	Mean ± SE	Minimum age	Maximum age
Male	21	41.86±3.69	16.00	67.00
Female	19	40.63±3.24	16.00	64.00
Total	40	41.27±2.44	16.00	67.00

The most frequent patients on study population were between 26 – 45 years old and lowest patients were patients more than 60 years old as seen in (Fig 3.2).

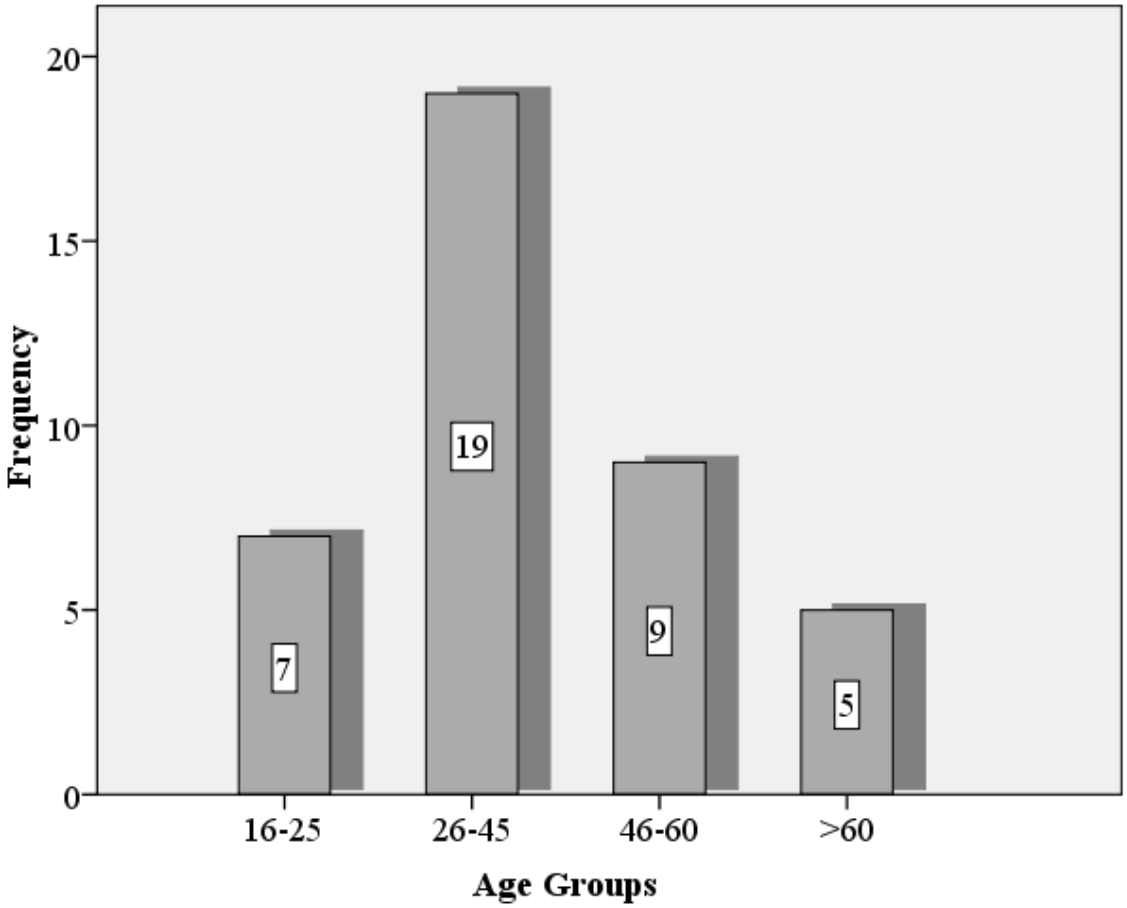

Fig 3.2 Frequency of age groups on study population.

Mean of serum ferritin level among AML patients was (431.86±16.61µg/L) which described in (Table 3.2). It was significantly higher than normal reference level.

Table 3.2 Mean serum ferritin level among AML patients on study population.

	N	Mean ± SE	Maximum level	Minimum level	P. value
S.Ferritin	40	431.86±16.61	613.6	243.5	.000

❖ Serum ferritin level was compared with normal reference level of (20-250 µg/L) (Burtis *et al*., 2005).

There was positively significant correlation between serum ferritin level and TWBCs among AML patients (r=0.345) (P<0.05). No significant correlation was found of serum ferritin level with age, Hb concentration and blasts% on study population (P>0.05) that shown in (Table 3.3).

Table 3.3 Correlation of serum ferritin with TWBCs, age, Hb concentration and blasts% on study population.

	Coefficient (r)	P. value
TWBCs	.345	.029
Age	.021	.900
Hb conc.	.084	.604
Blasts %	.062	.703

(Table 3.4) shows no statistical difference of serum ferritin level between FAB subclasses of AML, (P> 0.05).

Table 3.4 Mean serum ferritin level regard FAB subclasses of AML on study population.

FAB subclasses	N	Mean ± SE	P. value
M0	6	373.32±37.54	.107
M1	9	458.74±24.44	
M2	5	461.12±50.41	
M3	8	397.30±40.70	
M4	8	445.71±38.21	
M5	3	537.50±37.20	
M6	0	0	
M7	1	243.50	
Total	40	431.86±16.61	

There was no statistical difference of serum ferritin level among males and females with AML on study population (P. value > 0.05) as observed in (Table 3.5).

Table 3.5 Mean serum ferritin level regard to gender of AML patients participate in this study.

Gender	N	Mean ± SE	P. value
Male	21	454.15±26.39	.154
Female	19	407.22±18.40	
Total	40	431.86±16.61	

Chapter Four

Discussion, Conclusion, Recommendation

4.1 Discussion:

Ferritin is the principal iron storage protein participating in iron metabolism. As serum ferritin levels often reflect the amount of storage iron in the body, physicians have measured serum ferritin in order to evaluate iron deficiency or overload. Although a rise in serum ferritin concentration occurs in iron overload, hyperferritinemia without it has been reported in some inflammatory diseases and malignancies (Ota, 2000).

In this study, the mean serum ferritin level among AML patients was (431.86±16.61µg/L) which was higher than the normal reference value (20-250µg/L). This result agree with Jones *et al*., (1973) who reported a mean ferritin level (589±66 ng/ml), which was higher than the highest normal value (186 ng/ml). A similar result was obtained by Ahlawat *et al*., (1994) that ferritin in AML patients of (775 ng/ml) was significantly higher than control value (46.14 ng/ml) and did not show a decline with remission.

Abd El Maksoud *et al*., (2010) reported that significantly higher serum level of ferritin in newly AML diagnosed patients (1400.25ng/ml) and relapsed patients (1431.45ng/ml) compared with both control group (36.69ng/ml) and leukemic patients in remission stage (99.34ng/ml) ($P<0.001$).

All these previous studies suggested that serum ferritin concentration can be used for initial evaluation of AML disease which agreed with current study and Jacobs, (1984) disagree with this result, as he reported the present

evidences do not suggest that serum ferritin assay is useful either for specific diagnosis or monitoring of malignant diseases.

This study agrees with Tanaka and Kato, (1983) result that serum ferritin of patients with acute leukemia was markedly increased (698±447 ng/ml) with highest level shown of patients with acute monocytic leukemia (1156±324 ng/ml). In addition, Tanaka and Kato, (1983) reported markedly higher ferritin level content within leukemic cells (64.3±38.7 ng/cells) compared with ferritin in normal leucocyte (7.1±2.8 ng/cells), blasts from patients with acute monocytic leukemia has shown the highest level (105±26.6 ng/cells).

This finding is confirmed by Parry *et al.*, (1975) report which showed increased serum and white cells ferritin concentration in untreated patients. In remission patients have ever higher level. In contrast, leucocyte ferritin concentration decreased during remission. Mean serum ferritin in patients of AML at presentation was (1278±367µg/L) and that in remission was (1567±331 µg/L), mean leucocytes ferritin level at presentation was (89.6±26 fg/cell) and (25.7±10.8 fg/cell) in remission, the whole group was significant ($p<0.05$). His report suggested that the increased capacity for ferritin synthesis shown by myelogenous cells from patient with AML suggested that these cells themselves were the source of the increased amount of circulating protein. Parry *et al.*, (1975) have also shown that the increase in circulating ferritin during chemotherapy could be due to an increased release from damage leukemic cell and this increased the serum ferritin level.

In the present work serum ferritin value correlated positively with TWBCs ($r=0.345$) ($P=0.029$), this result agree with Abd El Maksoud *et al.*, (2010) and Jones *et al.*, (1973) result that serum

ferritin level correlated positively with TWBCs with coefficient (r = 0.69) and (r=0.49, P<0.005) respectively and disagree with result of Ahlawat *et al.,* (1994).

In this work, serum ferritin level among patients with AML did not correlated with both Hb concentration (P=0.604) and blasts % (P=0.703) which agreed with Ahlawat *et al.,* (1994) and Jones *et al.,* (1973) results and disagreed with Abd El Maksoud *et al.,* (2010) who reported inversely correlation of serum ferritin level with Hb concentration (r= -0.44) and positively with % of blast cells in bone marrow (r= 0.79).

In a previous report Aulbert and Fromm, (1987) demonstrated that extremely high serum ferritin in patients with immature AML (M1) and AML (M2) FAB classification before treatment. The mean ferritin concentration of AML (M1) was (2235 ±870 ng/ ml) and for AML (M2) was (1355±532 ng/ml). However serum ferritin level in AMML and AML (M4) and AML (M5) found to be significant lower (450±252 ng/ml). This finding disagreed with this study result which was no significant variation between FAB subclasses regarded to serum ferritin level among AML patients (P=0.107), that could be because of inappropriate distribution of FAB subclasses on study population.

There was no significant difference between males and females patients with AML (P=0.154) and no variation among age (P=0.90) in regard to serum ferritin level, this finding agreed with Ahlawat *et al.,* (1994) report.

4.2 Conclusion:

- Serum ferritin level among AML patients was significantly increased than normal reference value (P=0.000). Thus, Serum ferritin level can contribute in initial evaluation of AML disease.
- A positive correlation between serum ferritin level and TWBCs (r=0.345) (P=0.029) and non significant correlation with Hb concentration (P=0.604) were found.
- Non significant differences in serum ferritin level among FAB classification subclasses (P= 0.107).
- Non significant variation in serum ferritin level according to age (P= 0.90) and between males and females patients with AML (P=0.154).
- Non significant correlation of serum ferritin level with blast % (P=0.703).

4.3 Recommendation:

- More studies should be conducted to verify the status of body iron store and intracellular ferritin level.
- More variants should be included in future studies such as duration of the disease, liver involvement, remission status and blasts count.
- More studies should be performed to investigate other diseases associated with high serum ferritin concentration.

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Appendices I:

Sudan University of Science and Technology

College of Graduate Studies

Measurement of Serum Ferritin Level among

Acute Myeloblastic Leukemia Patients - Khartoum State

Date: \ \ 2014

Name:.....….....

Age: …….

Gander: male ☐ female ☐

Leukocyte count:……

Hemoglobin level:……..

FAB subclass:.....

% of blasts:…….....

Do you have any of these diseases?

- ✧ Iron deficiency anemia. Yes☐ No☐
- ✧ Thalasiemia Yes☐ No☐
- ✧ Anemia of chronic disease. Yes☐ No☐
- ✧ Sidroblastic anemia. Yes☐ No☐
- ✧ Hodgkin's disease. Yes☐ No☐
- ✧ Ovaries, Breast, lungs and esophagus cancer Yes☐ No☐
- ✧ Iron overload. Yes☐ No☐
- ✧ Renal diseases. Yes☐ No☐
- ✧ Other:.....

Ferritin level:……

No.....

Appendices II:

جـــــامعـة الـسودان للعـــلوم والتكـــنولوجـيا

كلــية الـــــدراســات العـــليا

برنامج ماجستير علوم المختبرات الطبية- تخصص أمراض دم

إسم المريض:.....

سوف يتم سحب عينة دم من الوريد بمقدار 2.5 مل بواسطة حقنة الطعن, بطريقة آمنة وغير مؤذية مع مراعاة جميع وسائل السلامة المعملية المتبعة لسحب العينات, قد يشعر المريض بوخز خفيف وانتفاخ موضعي في مكان الطعن يزول بعد فترة زمنية بسيطة و لا يسبب أي مضاعفات للمريض. وستستخدم عينة الدم في أغراض البحث العلمي فقط.

❖ **أوافق أنا المذكور أعلاه على أخذ عينة الدم لإجراء الدراسة.**

التوقيع:.....

التاريخ:.....

Appendices III:

Picture show Biosystem chemistry analyzer A15 (Barcelona, Spain).

Appendices IV:

COD 22934 2 x 40 mL + 2 x 20 mL
STORE AT 2-8°C
Reagents for measurement of ferritin concentration Only for *in vitro* use in the clinical laboratory

FERRITIN

CE

FERRITI
LATE

PRINCIPLE OF THE METHOD

Serum ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies. The agglutination of the latex particles is proportional to the ferritin concentration and can be measured by turbidimetry[1].

CONTENTS AND COMPOSITION

A. Reagent. 2 x 40 mL. Glycine buffer 170 mmol/L, sodium chloride 100 mmol/L, sodium azide 0.95 g/L, pH 8.2.

B. Reagent. 2 x 20 mL. Suspension of latex particles coated with anti-human ferritin antibodies, sodium azide 0.95 g/L (Note 1).

STORAGE

Store at 2-8°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: absorbance of the blank over 1.600 at 540 nm.

AUXILIARY REAGENTS

S. Ferritin Standard. For 1 x 3 mL (BioSystems Cod. 31127). Human serum. Ferritin concentration is given on the label. The concentration value is traceable to the Biological Reference Material WHO 94/572 (National Institute for Biological Standards and Control, NIBSC).

Human serum used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the standard should be handled cautiously as potentially infectious.

Reconstitute with 3.00 mL of distilled water. Stable for 1 month at 2-8°C.

Calibration curve: Prepare dilutions of the Ferritin Standard using 9 g/L saline as diluent. Multiply the concentration of the Ferritin Standard by the corresponding factor indicated below to obtain the ferritin concentration of the dilutions.

DILUTION	1	2	3	4	5
Ferritin Standard (µL)	30	60	120	180	240
Saline (µL)	210	180	120	60	—
Factor	0.125	0.25	0.5	0.75	1.0

REAGENT PREPARATION

For some automatic analyzers, the monoreagent modality can be used.

Working Reagent: Pour the contents of a Reagent B vial (Note 1), into a Reagent A bottle. Mix thoroughly. Stable for 20 days at 2-8°C. Smaller Working Reagent volumes can be prepared by mixing: 1 mL of Reagent B + 2 mL of Reagent A. Shake the Reagent B vial before pipetting.

Reagent open and kept in the refrigerated compartment of the analyzer is stable 2 months.

SAMPLES

Serum collected by standard procedures. Hemolyzed or lipemic samples are not suitable for testing.

Ferritin in serum is stable for 7 days at 2-8°C.

REFERENCE VALUES

Serum[2,3]

Children: 7-140 µg/L
Men: 20 - 250 µg/L
Women: 20 - 200 µg/L

This range is given for orientation only; each laboratory should establish its own reference range.

CALIBRATION

It is recommended to use a ferritin standard.

A calibration is recommended at least every 2 months, after reagent lot change or as required by quality control procedures.

ASSAY PARAMETERS

These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.

- Wavelength: 540±20 nm
- Temperature: 37°C
- Analysis mode: 2 points

	Blank	Sample / Standard
Sample / Standard	-	10 µL
Distilled water	10 µL	-
Reagent A	200 µL	200 µL

Mix and incubate 3 - 5 minutes. Then add:

Reagent B	100 µL	100 µL

Mix and read the absorbance after 10 seconds and after 5 minutes.

QUALITY CONTROL

It is recommended to use the Protein Control Serum levels I (cod. 31211) and II (cod. 31212) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 4 µg/L ferritin

– Measurement interval: (approximate depending on the standard concentration): 4-500 µg/L. For higher values dilute sample 1/5 with 9 g/L NaCl and repeat measurement. Linearity may considerably vary depending on the instrument used.

– Repeatability (within run):

Mean concentration	CV	n
61 µg/L	2.2%	20
145 µg/L	1.6%	20

– Reproducibility (run to run):

Mean concentration	CV	n
61 µg/L	3.7%	25
145 µg/L	1.6%	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Zone effect: > 30,000 µg/L.

– Interferences: Hemoglobin (10 g/L), lipemia (triglycerides 5 g/L), bilirubin (62 mg/dL) and rheumatoid factors (520 IU/mL) do not interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Ferritin is the major iron storage compound in the body. It consists of a protein shell enclosing a core of a variable amount of iron. Ferritin is present at particularly high concentrations in liver, bone marrow and spleen.

The plasma ferritin is in equilibrium with body stores and variations in the quantity of iron in the storage compartment are reflected in plasma ferritin concentration.

Serum ferritin concentration declines very early in the development of iron deficiency and it serves as a very sensitive indicator of iron deficiency. On the other hand, a large number of chronic infections, chronic inflamatory disorders (rheumatoid arthritis, renal disease) and malignancies (lymphomas, leukemias, breast cancer, neuroblastoma) result in increased serum ferritin concentration. Plasma ferritin is also increased in patients with hemosiderosis or hemochromatosis[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Shake the Reagent B vial gently before using.

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