Chapter One

Introduction and literature review

1.1 Introduction:

Chronic myetogonous (or myeloid) leukemia (cml) also known as chronic granulocytic leukemia (CGL) is a cancer of the white blood cells. This clonal myeloproliferative disorder characterized by increased and un regulated growth of predominantly myeloid cells in the bone marrow and accumulation of these cells in the blood. CML is clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils) esoinophils and basophils and their precursors is the main finding. It is a type of myeloprolifrative disease associated with a characteristic chromosomal translocation called Philadelphia chromosome (Provan *et al*., 2004).

CML accounts for around 15% of leukemia and may occur at any age. The disease occurs in both sex male and female. Most patients are diagnosed in the chronic phase. Many patients are asymptomatic and are diagnosed when an elevated white count is found on a routine complete blood count (CBC).The peripheral blood picture shows extremely high WBCs count with the whole spectrum of neutrophilic cell development (Hoff brand *et al*., 2006).

1.2. Literature review:

1.2.1 Blood: definition and composition:

Blood is considered a specialized form of connective tissue given its origin in bones and the presence potential molecular fibers in the form of fibrinogen. Blood is a specialized body fluid that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products any from these same cell. It is composed of blood cells suspended in a liquid called bleed plasma (Sukkar *et al.,* 2002).

Plasma which constitutes 55% of blood fluid and contains dissipated proteins, glucose, mineral irons, hormones, carbon dioxide. Albumin is the main protein in plasma, and it functions to regulate the osmotic pressure of blood. The blood cells are mainly red blood cells and white blood cells, including leukocytes and platelets, the most cells in vertebrate blood are red blood cells. These contain hemoglobin; an iron-containing protein, which fabricates transportation of oxygen by reversibly binding to this reversibly binding to this respiratory gas. The blood is circulated around the body through blood vessels by the pumping action of heart (Bruce and Albert, 2008).

1.2.2. Hematoposies:

Hematoposies is process whereby blood cells are made. Site of haemopiesis in the first few weeks of gestation the yolk sac is the main site of these common precursors site of haemopiesis. These common precursors of endothelial and heamopoietic cells are believed to seed the liver, spleen and bone marrow and from 6 week until 6-7 month of fetal life the liver and spleen are the major heamopoietic organ and continue to produce blood cells until about 2 week after birth the bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is only source of new blood cell (Hoffbrand *et al*., 2006).

1.2.3. Erythrocyte:

Is a mature red cell, it is mainly composed of hemoglobin surrounded by a flexible protein membrane and other lipid bi layer. The biconcave form of the red cell and membrane which is made is made of specialized deformable protein fibers enable the cell to pass through capillaries of small diameter its shop also provides a large surface area for the exchange respiratory gases (Chess Brough and Monica, 2006).

1.2.4. Erythropoiesis:

Erythropoiesis passes from the stem cell through the progenitor cells colony-forming unit granulocyte, erythroid, monocyte and megakaryocyte (CFUCEMM), burst forming unit (CFUE) to the first recognizable erythrocyte precursor in the bone marrow, the pronormoblast. This is large cell with dark blue cytoplasm, the promoroblast gives rise to a series of progressively smaller nosmoblasts blast a number of cell divisions. They also contain progressively more hemoglobin in the cytoplasm (Hoffbrad *et al*., 2006).

1.2.5.Hemoglobin structure and synthesis:

The hemoglobin molecule consist of two primary structures:

Hemeportion, this structure involves four irons atoms in ferrous state (Fe^2) because iron in the ferric state F^3 can not bind oxygen surrounded by protoporphyrin, the protoporphyrin is the final product in synthesis of hememolecule.

Globinportion: These consist of amino acids linked together to form a polypeptide chain. The most significant chains for adult hemoglobin's are the alpha and beta chains. Alpha chains have 141 amino acids in unique arrangement , and beta chains have 146 amino acids.

An additional structure that supports the hemoglobin molecule is 2.3 diphosphoglycerate (2.3-DPF). This structure is intimately related to oxygen affinity of hemoglobin (Betty Cielsa 2007).

1.2.6. Anemia:

Anemia is a medical condition which the red blood cell count or hemoglobin is less than normal. For men, anemia is typically defined as hemoglobin level of less than 13.3 g/dl and in women as hemoglobin of less than 12.0 g/dl (William and Shiol, 2007).

Anemia is one of the major common blood disorders, occurs when the level of red blood cells in the body becomes too low. This can lead to health problems, because RBC contain hemoglobin, which carries oxygen to body's tissues. Anemia can cause variety of complication including fatigue and stress on body organs. (Hoffbrand *et al*, 2006)

1.2.6.1 Anemia classifications:

Anemia can be classified into two categories:

1- Morphological classification.

2- Etiological classification.

The main types of the anemia are recognized on the basis of the mean. (MCV), MCH, MCHC and main Hb Concentration .

A) Microcytic hypo chronic anemia:

Microcytic anemia in it the red blood cell are usually also hypochronic, meaning that the red blood cells appear paler that usual. This is reflected by a lower-than-normal. Mean corpuscular hemoglobin concentration (MCHCL, a measure representing the amount of hemoglobin per unit volume of fluid inside the cell, normally about 32-36 g/dl.For example iron deficiency anemia thalassemia .

B) Norm Chronic Normocytic Anemia:

It forms of anemia in which the average size and hemoglobin content are within normal range. Usually microscopic examination of the red cells shows them to much like normal cells anemia caused by the loss of blood.

C) Macrocytic Anemia:

Anemia characterized by the presence of abnormally large RBCs in the peripheral blood.

Some of causes of macrocytic anemia include nutritional deficiencies (vitamin B_{12} and Folate) for example megaloblastic anemia (Hoffbrand *et al.*,2006).

2- Etiological classification:

According to etiological the anemia can be classified into:

- Excessive destruction of RBC.

- Blood loss.
- Inadequate production of RBC. (William and Shiel 2007)

Anemia can be classified by cytometric schemes (those depend on the cell size and hemoglobin content parameter) such as MCV and MCHC) erytrokinetic scheme (those that take into account the rate of RBC production and destruction) and biochemical molecular scheme (those that consider the etiology of anemia of molecular level (William and Shiel 2007).

1.2.7.Platelet:

The normal platelet count is 150-400 × 10^9L and the life span is 9-10 day (Hoffbrand *et al.*, 2006).

1.2.7.1.Platelet production:

Are produced in the bone marrow by fragmentation of the cytoplasm megakaryocyte (Hoffbrand *et al.*, 2006).

1.2.7.2.Platelet function:

The main function of platelet is formation of mechanical plugs during the normal haemostatic response to vascular injury (Hoffbrand *et al.*, 2006).

1.2.8.White blood cells (WBCs):

White blood cells are divided into two broad groups. The phagocytes and the immunocytes. Granulocytes, which include three types of cells that is neutrophils, eosinophils and basophils together with monocytes comprise the phagocytes only mature phagocytic cells and lymphocytes are found in normal peripheral blood. The lymphocytes, their precursor cells and plasma cells, which make up the immunocytes population. In an adult there are about 4.0 – 11.0 /10^9 white cells per liter of blood (Hoffbrand *et al*., 2006).

1.2.8.1.Neutrophils:

They have characteristic dense nucleus consisting of between two and five lobes and a pale cytoplasm with anti regular outline containing many fine pink-blue or grey-blue granules. They have many shaped nucleus. The life span of neutrophils in blood is 6-10 h (Hoffbrand *et al*., 2006).

1.2.8.2.Eosinophil:

Are slightly larger than a neutrophils the nucleus of most eosinophils has two lobes , the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclear lobes. They have special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation (Hoffbrand *et al.*, 2006).

1.2.8.3.Basophil:

There are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granules which over be the nucleus and contain heparin and histamine. In the tissues they become most cells. They have immunoglobulin E

(IGE) attachment sites. The nucleus is usually bilobed. The normal basophil count is less than 0.1 O 10^9/L (Hoffbrand *et al.*, 2006).

1.2.8.4. Monocyte:

Largest of the circulating white cells and posses a large central oval or intended nucleus with clumped chromatin and abundant cytoplasm stains blue and contains many fine vacuoles. The monocyte precursors in marrow are

difficult to distinguish from myloblasts and monocyte (Hoffbrand *et al.*, 2006).

1.2.8.5. Lymphocytes:

The body produces the main type of lymphocyte to carry out specific immune responses.

T-Lymphocyte which form 56-80% of lymphocytes in blood with about two third being $(1)_4^+$ helper T cell and CD_8^+ cytotoxic T cell. .

B- Lymphocyte which form 10-30% of lymphocyte in blood.

Non-B, Non-T-lymphocyte make up to 2-10% of circularity lymphocytes , they include natural killer (N K) lymphocyte (Chess Brough and Monica 2006).

1.2.8.6. Functions of WBCs:

1) Defend against bacterial, fungal and parasitic infections. The body uses white blood cells to fight infection. The interesting thing is that there are several types of white blood cells used to fight different infection that is the neutrophils, which defend against bacterial and fungal infection.

2) The eosinophil cell deal with parasitic infections. If the eosinophil cell count in blood is high, it is an indication of a parasitic infection. This type of cell is also responsible for reacting to any substance that causes an allergic response.

3) Basophils : their granules contain heparin and histamine .In the tissue they become mast cells.

4) Monocytes:They leave the blood to the tissues after 20 40 hours. They may assume different functions in the different tissues.

5) The lymphocyte : are most responsible cells for providing the body with immunity. They create antibodies (Sueanne, 2012).

1.2.9.Leukemia:

1.2.9.1. Definition of Leukemia:

Leukemia "American English or British English) word is a type of cancer of blood or bone marrow characterized by an abnormal increases of immature white blood cells called (blasts) (Douglas and Anderson 2002).

1.2.9.2. Classifications of Leukemia:

No single known cause for any of the different types of leukemia exists . Leukemia like other cancers , results from mutation in the DNA. Certain mutation can trigger leukemia by activating oncogenes or deactivating tumor suppressor genes, and shore by distributing the regulation of cell death, differentiation or division. Those mutations may occur spontaneously or as a result of exposure to radiation or carcinogenic substances, few viruses such as human T-lymphotropic virus, and some chemicals, notably benzene and alkylating chemotherapy agents

for previous malignancies. Use for of tobacco is associated with a small increase in the risk of developing acute myeloid leukemia in adult. Some people have a genetic predisposition towards developing leukemia, so may be inheritery (Wiernik 2001).

1.2.9.3.Classification of Leukemia:

Clinically and pathologically leukemia is subdivided into a variety of large groups. The first division is between its acute and chronic forms. (Hoffbrad *et al*., 2006).

1.2.9.3.1.Acute leukemia:

Is characterized by rapid increase in the numbers of immature blood cells. Crowding due to such cells makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression accumulation of the malignant cell. Acute forms of leukemia are the most common forms of leukemia in children. (Hoffbrad *et al*., 2006).

1.2.9.3.2.Chronic leukemia:

Is characterized by the excessive build up of relatively mature, but still abnormal, white blood cells, typically taking months or years to progress. The cells are produced at a much higher rate than normal resulting in many abnormal white blood cells. (Harrison *et al.*, 2005)

Where a acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment ensure maximum effectiveness of therapy. Chronic leukemia mostly occur in older people, but can theoretically occur in any age group. In addition the diseases are subdivided according to which kind of blood cell is affected. This split divides leukemia into

myeloid or lymphocytic leukemia ; in the lymphocytic leukemia, the cancerous change take place in a type of marrow cell that normally goes on to form lymphocyte infection fighting immune system cells. Combining these two classifications provides a total of four main categories. Within each of these four main categories, there are typically several subcategories , finally , some rarer types are usually considered to be outside of this classification scheme (Harrison *et al*., 2005).

1.2.9.4.Acute lymphoblastic:

Most common type of leukemia in young children, this disease also affects adult especially those aged 65 and older. Standard treatments involve chemotherapy and radiotherapy. The survival rates vary by ages : 85% in children and 50% in adults Subtypes include precursors B acute lymphoblastic leukemia, precursors T acute lymphoblastic , Burkitt's leukemia, and acute biphenotypic leukemia (Harrison *et al*., 2005).

1.2.9.5. Chronic lymphocytic leukemia:

Most often affects adult over these of 55. It sometimes occurs in younger adults but it almost never affects children. Two-third of affected people are men, the five-year survival rate is 75% it is incurable, but there are many effective treatments.One subtype is B-cell pro-lymphocytic leukemia, a more aggressive disease (Harrison *et al*., 2005).

1.2.9.6. Acute Myelogenous leukemia:

Occurs more commonly in adult than in children and more commonly in men than women. AML is treated with chemotherapy, the five-year survival rate is

40%. Subtypes of AML include acute Promyolocytic leukemia acute myelocytic leukemia acute megakaryoblastic leukemia (Harrison *et al*., 2005).

1.2.9.7. Chronic myelogeous leukemia:

Occurs mainly in adult and a very small number of children also develop this disease. The five-year survival rate 90%. One subtype is chronic monolithic leukemia (Paula Moyer, 2006).

1.2.9.8. Variant of lymphocytic leukemia "less common":

1) Hairy cell leukemia (HCL)
2) T-cell prolymphocytic leukemia (T.P)
3) Large granular lymphocytic leukemia
4) Adult T-cell leukemia is caused by human T-lymphocytic virus (HTLV).(Elaine and Harris 2001).

1.2.9.9. Symptoms and signs:

Damage to the bone marrow, by way of displacing the normal bone marrow cells with higher numbers of immature white blood cells, results in a lack of blood platelets, which are important in the blood process. This means people with leukemia may easily become bruised, bleed excessively or develop pin prick bleed (petechiae). White blood cells, which are involved in fighting pathogens may be suppressed or dysfunctional. This causes the patient's immune system to be unable to fight off a simple infection some patients experience fragment infection, ranging from infected tonsils sores in the mouth, or diarrhea to life-threatening pneumonia or opportunistic infections. Finally, the red blood cell deficiency lead to anemia, which may cause dyspnea and pallor (Hoffbrand *et al.*, 2006).

1.2.9.10.Diagnosis:

Diagnosis is usually based on a complete blood count and a bone marrow examination following observations of the symptoms.However in rare cases blood test, may not show if a patient has leukemia usually this is because the leukemia is in early stages or has entered remission. A lymph node biopsy can be performed as well in order to diagnose certain types of leukemia in certain situations. Following diagnosis blood chemistry tests can be used to determine the degree of liver and kidney damage or the effects of chemotherapy on the patient (Pasmant *et al.*, 2009).

1.2.9.10.1 Complete Blood Count (CBC)

CBC is a test used as abroad screening test to check disorders such as anemia, infection, leukemia and many other diseases. It is actually a panel of tests that examines different parts of the blood and includes the following:

- Hemoglobin measures the amount of oxygen carrying protein in the blood.
- White Blood Cells (WBCs) count is a count of the actual number of white blood cells per volume of blood. Both increases and decreases can be significant.
- White blood cells differential looks at the types of white blood cells present.
- Red blood cells (RBCs) count is a count of the actual number of the red blood cells per volume of blood both increases and decreases can point to abnormal conditions.
- Hematocrit (PCV) measures the percentage of red blood cells in a given volume of whole blood.
- Mean corpuscular volume (MCV) is measurement of the average size of RBCs.
- Mean corpuscular hemoglobin (MCH) is a calculation of the average amount of oxygen carrying hemoglobin inside a red blood cell.
- Mean corpuscular volume concentration (MCHC) is a calculation of the average concentration of hemoglobin inside a red cell.
- Platelets count is the number of platelets in a given volume of blood. Both increases and decreases can point to abnormal conditions of excess bleeding or clotting. (Lichtman, 2003)

1.2.9.10.2 Bone marrow examination:

Bone marrow examination refers to the analysis of samples of bone marrow aspiration. Bone marrow examination is used in the diagnosis of a number of conditions including leukemia, multiplemyeloma, lymphoma, and anemia. (Lichtman, 2003)

1.2.10. Chronic myeloid leukemia:

1.2.10.1. Definition of Chronic myeloid leukemia:

Is a cancer of white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is the main finding. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome. The Ph chromosome is an abnormally short chromosome 22 that is one of the two chromosomes involved in a translocation can exchange of material with chromosome g-this translocation takes place in a single bone marrow cell and through the process of clonal expansion (the production or many cells from this one mutant cells) it gives rise to the leukemia (Hoffbrand *et al.*, 2000).

1.2.10.2. Pathophysiology:

CML was the first malignancy to be linked to to a clear genetic abnormally, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because was first discovered and described in 1960 by two scientists from Philadelphia.In this translocation parts of two chromosomes (the 9^{th} and 22^{nd} by conventional karyotopic numbering) switch places. As a result , part of the BCR (break point cluster region come gene from chromosome 22 is fused with the ABL gene on chromosome 90 this abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight. (P210 is short for $210K_1$) a protein a short hand used for characterizing proteins based solely on size. Because obi carries a domain that can add phosphate groups to tyrosine residues (atyrosine kinase) , the BCR- able fusion gene product is also atyrosine kinase. (Hehlman *et al.,* 2007)

The fused BCR-ABL protein interact with the interleukin 3 beta receptor subunit. The BCR – ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the call cycle, speeding up cell division, moreover, the BSR-ABL proteins inhibit DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities the action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia.

The ph chromosome is also found in form of a cut lymphoblastic leukemia (ALL) (Hehlman *et al*., 2007).

1.2.10.3. Types of chronic myeloid leukemia:

1.2.10.3.1 Adult chronic myeloid leukemia:

1- Chronic Myeloid leukemia (ph+ve) Philadelphia chromosome is a specific chromosomal abnormality that is associated with chronic myelogenous leukemia. It is result of translocation between chromosome 9 and 22. The presence of this translocation is sometimes used to test for CML. Since 95% of people with CML have this abnormality (Colon *et al*., 1999).

2- Chronic myeloid leukemia (ph-ve) less than 5% patients with features suggestive of CML are negative for the Philadelphia chromosome and BCR-ABL translocation. These patients, usually have some of the hematological features typical of myeliodysplasia, the prognosis appears to be worse than for ph-positive CML. (Colon *et al*., 1999)

3- Juvenile chronic myeloid leukemia this rare condition affects young children and has characteristic clinical features including skin rashes, lymphodenopathy, hepatosplenomegaly and recurrent infection the blood film shows monocytosis. A high hemoglobin F level is a useful diagnostic feature, and the prognosis is poor. (Ching *et al.*,1999).

4- Chronic esoinophilic leukemia, these are rare conditions in which there is a relatively pure proliferation of mature cells. Some patients with idiopathic hypereosinophilia have an interstitial deletion of chromosome and general the prognosis is good. (Hoffbr *et al.*, 2006).

5- Chronic myelomonocytic leukemia, this constituted approximately 20% of cases of MDS when it was classified with them. It is defined by a persistent monocytosis. The total white cells count is usually raised, the patients may

develop skin rashes and around half of them have splenomegaly lymphadenopathy may also be present treatment is difficult. (Hoffbrand *et al.*, 2006).

The children type Juvenile myelomonocytic leukemia this present in the first 4 years of life and has features of both myelodysplasia and myeloproliforative disease. There is often skin rash, hepatosplenomegaly and lymphadenopathy. There is monocytosis clonal cytogent changes but not the BCR –ABL fusion gene (Hoffbrand *et al*., 2006).

6- Chronic neutrophilic leukemia (CNL) is a rare myeloproliforative disorder that features a persistent neutrophilia in peripheral blood, myeloid hyperplasia in bone marrow hepatosplenomegaly, and the absence of the Philadelphia chromosome. (Hoffbrand *et al.*, 2006)

1.2.10.4. Symptoms and signs:

Patients are often asymptomatic at diagnosis, presented incidentally with elevated white blood cell count on a routine laboratory test. In this setting, CML must be distinguished from a leukemia reaction, which can have a similar appearance on blood smear. Symptoms of CML may include enlarged spleen causing pain on the left side , malaise, joint and /or hip pain , low-grade fever, increased susceptibility to infections, anemia and thrombocytopenia with easy bruising , although an increased platelet count “thrombocytosis” may also occur in CML. (Hoffbrand *et al.*, 2006).

1.2.10.5. Diagnosis:

CML is often suspected on the basis of the complete blood count, which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased this feature may help in differentiating CML from a leukemoid reaction. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by detecting the Philadelphia chromosome by routine cytogenetic, by fluorescent *in situ* hybridization or by PCR (Tefferi, 2006).

1.2.10.6. The biochemical changes:

The biochemical changes that are seen in CML are non-specific. Patients diagnosed in chronic phase may have a slightly raised serum uric acid but the level is frequently normal. The serum alkaline phosphates is usually normal or slightly raised. The lactate dehydrogenize (LDH) is usually raised. Serum K+ may be raised due to leakage of intracellular potassium from platelets.

The serum vitamin B_{12} and B_{12} binding capacity are greatly increased due to raised level of transcobalamin-1 in transformation.

The serum uric acid may be raised and tests of liver function are usually moderately abnormal. Hypercalcaemia is present and usually due to bone destruction (Hoffbrand *et al.,* 2006).

1.2.11 Hematological change in CMC:

The Hematological changes in CML are as follow:

1- Leucocytosis is usually > 50 $x10^9$/L and sometime > 500 $x10^9$/L. a complete spectrum of myeloid cells seen in the peripheral blood . the level of nutrophils and mylocytes exceed those of blast cells and promylocytes

2- Increase circulating basophils
3- Normochronic normocytic anemia 9 as usual.
4- Platelet count may be increased (most frequently) , normal or decreased.
5- Bone marrow is hyper cellular with granulopoietic predominance. (Hoffbrand and Moss, 2006).

1.3 Previous studies:

In Nigeria Akani *et al* (2010) reported significantly high values for RBCs,hemoglobin ,PCV ,MCV,MCHC,neutrophils ,lymphocytes and ferritin level in CML patients compared with the control group.They attributed this to a rise in the plasma concentration of interleukin-6. The same authors registered numerically higher WBCs , platelets count and eosinophils % among CML patients than the control group.

Another study was performed in Nigeria by Nwannadi *et al* (2011),they found that CML is more common in males than in females ,the mean age at diagnosis of the disease was 35.2±2.8 years. They observed that the most frequent symptoms were weakness ,followed by fever, weight loss and bone pain.PCV was found to be reduced below the reference range , WBCs were markedly elevated while platelets count was normal.

Kaur *et al* (2010) investigated the hematological profile in CML Indian patients. They found that the majority of the patients were anemic and had increased leuococytosis. They observed different types of immature cells and basophilia in all CML while eosinophilia in 55% of the study group.

1.4. Rational:

Leukemia is major public health problem in Sudan, it has great impact on both individual and society.

Also leukemia is associated with significant morbility and mortality. CML might cause many compilications including anemia, bleeding , neutropenia and other compilications.

The study is conducted to obtain clear information about the hematological parameters on CML patients.

1.5. Objective

1.5.1. General Objective:

To find the Measurement of Complete Hematological Parameter in Chronic Myeloid Leukemia Patients attended in Radiation and Isotope Centre

1.5.2. Specific Objectives:

1. To estimate total and differential leukocyte in CML patient and compare with the control group.
2. To estimate RBCs count and RBC indices in CML patients and compare with the control group.
3. To estimate platelet count and compare with the control group.
4. To estimate immature blood cell in CML patients and compare with the control group.

Chapter Two

2. Materials and methods

2.1. Study design

This is a case-control study. The study was carried out at radio isotopic center at Khartoum (RICK)

2.2. Study population

The study population is Sudanese has CML

2.3. Sampling and sample size

100 samples, 50 samples as case and 50 as control were selected

2.4. Data collection

Specifically designated questionnaire and blood samples were collected by standard method and analysis for specific parameters was used as tools of data collection

2.5. Data processing

All data included questionnaire was coded and listed in the master sheet and then computerized

2.6. Statistical analysis

Qualitative data was presented in form of pies. Data were presented as means. Separation between the means was performed by independent-sample student T Test using **S**tatistical Package for **S**ocial **S**cience (SPSS16.0).

2.7. Laboratory procedure

Venous bloods were collected as follows:

- The site of collection was disinfected using alcohol.

- The vein located (sometimes tourniquet) was used to locate the vein.

- The blood was slowly poured in EDTA container and mixed gently and CBC was determined

2.7.1. Complete blood count

An automated hematology analyzer system was used (sysmex KX-21N)

2.7.1.1 Principle of automated hematology analyzer system (sysmex) :-

In white cell and red cell transducer channel chambers the white cell and RBC in sample are separated through the different apertures and are counted by using electronic resistance (Impedance) detection for counting and sizing cells two unique feature enhance the impedance technology . in the blood cell platelet channel sheathed stream with hydrodynamic focusing is used to direct the cell file in single file through the aperture there by reducing coincident passage and pulse height irregularities and in both the WBCs and RBCs , platelet channels (floating threshold) are use to discriminated each cell population , the signals are transmitted in sequence to analog circuit and then to particles size distribution analysis circuits for conversion to cumulative cell size distribution data , particles size distribution curves constructed ,and the auto discrimination level in then set by the microprocessor for each cell population for example the lower platelet threshold maybe set between 2-6 FL and upper from 12-30 FL based on the particles size distribution, this floating threshold criteria allow for discrimination of cell

population as sample basis of cell count include the pulses between the lower and upper auto discrimination level , upper general count

In the RBCs channel the flooding discrimination is particularly useful in separating platelets from RBCs in the Hb flow cell ,the concentration of cyanmethemoglobin is measured as absorbance at 540 nm from the Hb concentration

2.7.2. Procedure:-

1- The reagent needed was checked for expiry date before use.

2- The power switch turned on, self auto rinse and back ground check was automatically performed the vend (vend for analysis) appear. samples NO is input by pressing (sample NO) and the number of the sample was entered and the (Enter) key was pressed.

3- Samples were mixed sufficiently and the tube was sited to the sample probe , and in that condition the (start) switch was pressed , when the LCD screen display had been (analysing) the tube was removed.

4- The result was displayed in the LCD screen and printed out.

2.7.3. Differential white blood cell count:-

2.7.3.1 Thin blood film preparation:

The differential leukocyte count was done by spreading a drop of well mixed blood placed on a clean stationary slide, thin blood film was made manual proper.

2.7.3.2 Staining method of thin blood film:

Thin films were air drained, then stained after fixation with ethanol , and examined under microscope by using an oil immersion and 100 X magnification one hundred white cell were counted and identified as either neutrophil , basophil , lymphocytes ,band , monocyte , eosinophil and any typical or immature cells also were counted . The percentage of each cell type was reported.

2.8. Ethical consideration

The study was approved by the ethical committee of the college Medical Laboratory Sciences, Sudan University of Science and Technology. Verbal consent was taken from all the participant after they had been informed about the procedure of the blood collection and the aim of study.

Chapter Three

3. Results

3.1. Distribution of fifty CML patients according to gender and age:

Distribution of fifty CML patients according to gender and age are displayed in figures 1 and 2 respectively. Males represent 68% while females represent 32% of the studied CML patients .The highest occurrence of CML (30%) was found in adults aged 40-49 years and the least occurrence was in the children less than 20 years (6%) and older more than 70 years (5%)**.**

3-2 The effect of CML on the leukocytes total ,differential and platelets count:

This is displayed in Table 3.1

CML patients exhibited significantly ($P<0.05$) higher total white blood cells count ($154.2x10^3$) than that of the control group ($5.72x10^3$).

A significant increase in the esinophil (5.04%) and basophil (4.0%) percentage was found in CML patients when compared with that of the control group (2.7% , 0.18%).

Chronic myeloid leukemia caused a significant reduction in the values of the neutrophils (37.2%), lymphocyte (5.72%) , monocyte (3.32%).

Also a significant increase ($P<0.01$) in the platelet count was observed in CML patients ($335.7x10^3$) when compared with that of the control ($274.6 x10^3$)

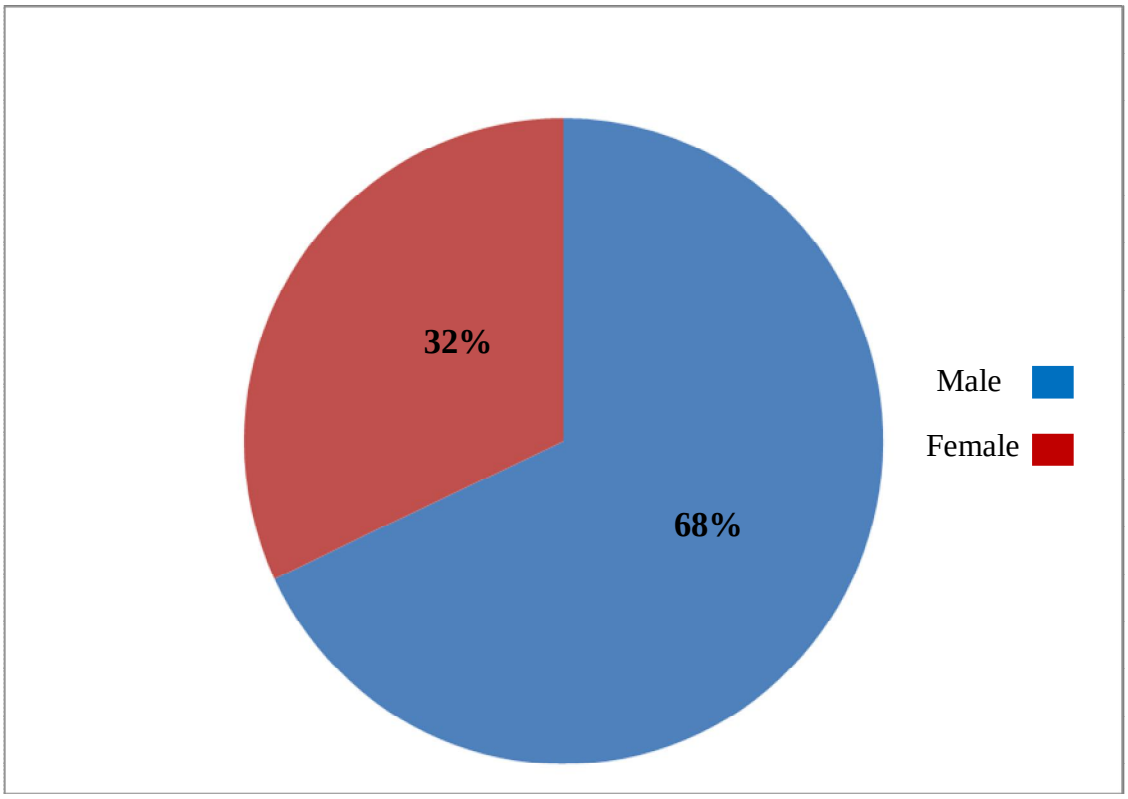
3-3The effect of CML on the RBCs count and indices:

Table (3-2) shows that CML patients exhibited significantly ($P<0.05$) lower RBCs count ($2.09x10^6$) and hemoglobin level (9.35g/dl) than that of the control group .The patients exhibited numerically lower PCV% (27.64±7.2) than that of the control group (39.8±4.75).

CML caused insignificant increase, between the patients and the control group, with regard to the values of MCV (89.8±9.41flcompared to 78±8.48fl),MCH (30.18±6.1pg compared to 28.92±4.42pg) and MCHC (33.4±5.61g/dl compared to 32±0.95g/dl) Table (3.2)

Figure (3) shows that:

Figure (3) shows the percentage of immature cells found in the blood of the study group.



Figure(3-1) Distribution of the CML patients according to gender

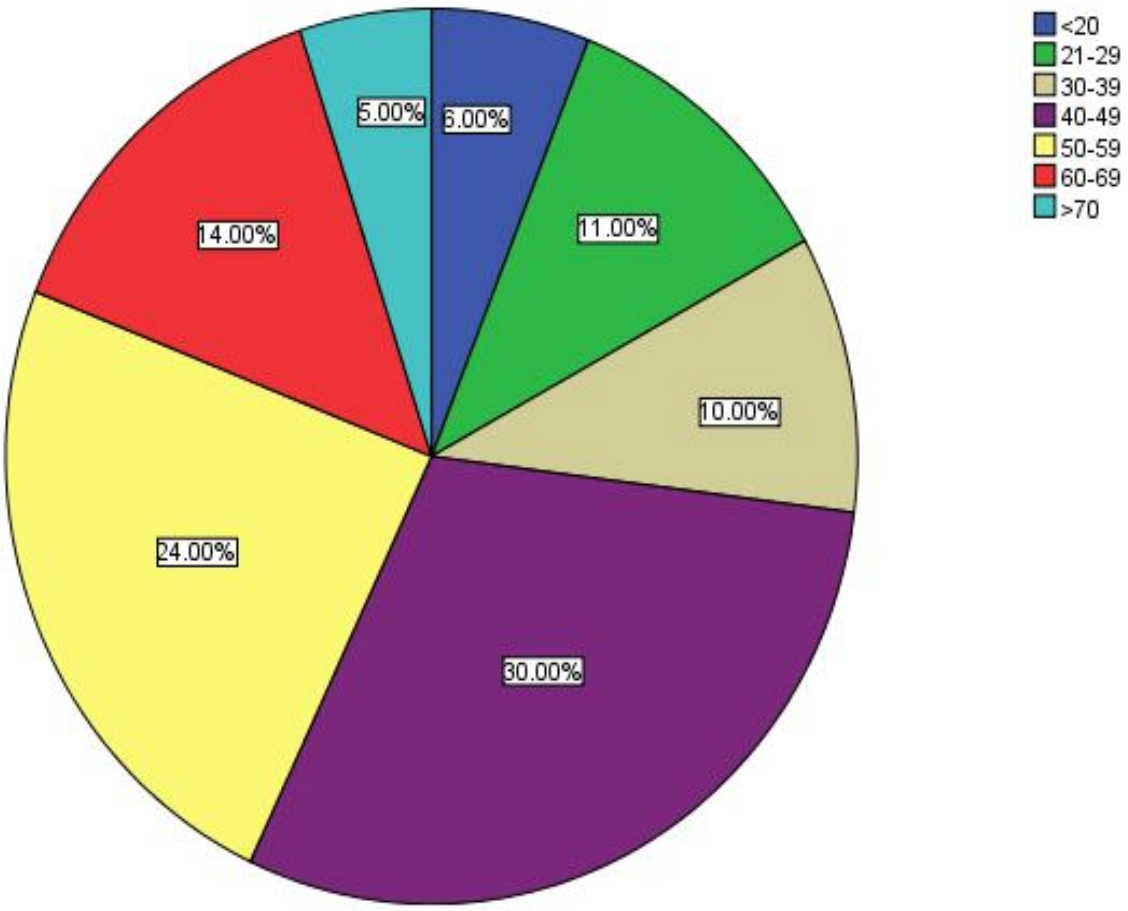


Figure (3-2) Distribution of CML patients according to age

Table (3-1): The effect of CML on the WBCs total and differential count and PLT' count

Parameters	Control M± SD.	Patient M±SD	Significance Level
TWBs × 10^3	5.72 ± 1.83	154.22 ± 83.9	**
Neutrophil (%)	57.9 ± 10.33	37.2 ± 14.7	**
Monocyte (%)	6.3 ± 2.3	3.32 ± 0.5	**
Lymphocyte (%)	33.9 ± 10.16	5.72 ± 4.3	**
Esinophil (%)	2.7 ± 0.3	5.04 ± 1.7	**
Basophil (%)	0.18 ± 0.03	4.0 ± 0.51	**
PLTs x 10^3	274.62 ± 74.9	335.7 ± 124.03	N.S

Number: 50 patients and 50 control

N.S: Not significant at ($P \leq 0.05$)

****:** P value significant at ($p \leq 0.01$)

SD: Standard deviation

M: Mean

Table (3-2): The effect of CML on RBCs count and indices

Parameters	Control M± SD.	Patient M±SD	Significance Level
RBC × 10^6	4.73 ± 1.6	2.09 ± 0.7	**
Hb g/dl	13.00 ± 1.56	9.35 ± 2.01	**
PCV (%)	39.80 ± 4.75	27.64 + 7.21	N.S
MCV FL	78.0 ± 8.48	89.82 ± 9.41	N.S
MCH pg	28.92 ± 4.42	30.18 ± 6.1	N.S
MCHC g/dl	32 ± 0.95	33.4 ± 5.61	N.S

Number: 50 patients and 50 controls ****:** P value significant at ($p \leq 0.01$)

N.S: Not significant at ($P \leq 0.05$)

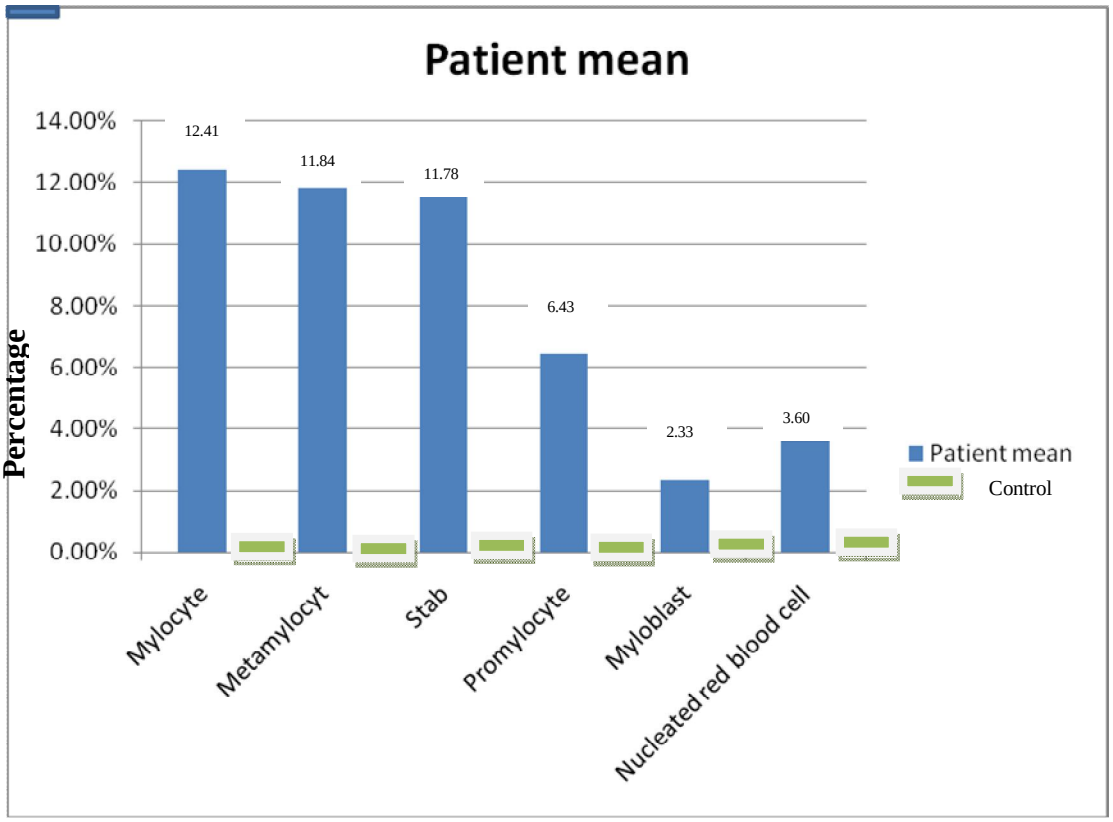


Figure (3.3): Percentage of immature WBCs and nucleated RBCs among the study population

Chapter Four

4. Discussion, Conclusion and Recommendation

4.1 Discussion:

The American Cancer Society reported that leukemia is the sixth leading cause of cancer death among men and the seventh cause of cancer death among females. In Nigeria Akanni et al (2010) found the distribution of 33 CML patients according to the sex to be 54.5 % males and 45.5% females which is 1.2:1. Nwannadi, *et al.*, 2011 reported higher incidence of CML in males than in females. These studies are on line with the current work that males are more susceptible to the disease than females. In the current work the ratio of males to females is 2.01:1 which is higher than that found by Akanni et al (2010).Men are more prone to the disease than women and this may be due to men are more predisposed to the causes and risk factors for chronic myloblastic leukemia that is nuclear radiation, X ray, and chemicals like carcinogenic drugs, cobalt, asbestose etc.

Dickstein et al (1993) found the disease to occur in all age groups but the median at the diagnosis was somewhere between 39 -48 years ,Kaur et al (2012) reported the medium age for CML in India to be 39-49 years. Both theses studies' findings are comparable to the finding of this study that is 40- 49 years.

Akanni et al (2010) reported leuococytosis ($21.07x10^3$ /µ l±41.57) in Nigerian CMLpatients. A high leuococytosis level($547x10^9$/L± was found in India by Kaur et al (2012).The findings of these two studies fortify the results of this work. Also Hoffbrand () described leukemia as an uncontrolled proliferation of the white blood cells which is on line with the leuococytosis found in the current work.

In this study CML patients showed an increased count of eosinophils and basophils which is on line with the observations of Tefferi (2006) that eosinophilia and basophilia are universal in CML patients. Kaur et al (2012) reported eosinophilia, basophilia, anemia and blasts in CML patients which accords with the findings of the present work.

Akanni et al (2010) reported higher values than of the this work for neutrophils (44.45±2.8) %,lymphocytes (52.82±20.42)%, erythrocytes ($3.97x10^6$/μL) and hemoglobin(11.92g/dl).The same authors reported lower values than of this study with regard to eosinophils(1.71±0.7)%,platelets ($254.81x10^3$/μL) and they did not observe any basophils or immature cells. The discrepancy between the findings of Akanni et al (2010) and the current work can be attributed to variation in the stage and duration of the disease , kind of treatment, and/ or feeding habits.

Sawyers (2010) reviewed CML and reported that CML is a malignant clonal hematopoietic disorder that results in an increase not only of the myeloid cells but also of the platelets; which is consistent with the presence of a defect in a pluripotent hematopoietic stem cell.

Halboob *et al,* (2006) observed immature leucocytes in CML patients which is on line with the results of this work. In the current work immature cells represents (44.80%) of the leucocytes differential count. The appearance of these immature cells was attributed to the abnormal differentiation of the leukemic cells (Metcalf 1971). Also CML was defined as an abnormal increase of immature white blood cells called (blasts) (Douglas and Anderson 2002).

4.2. Conclusion:

This study included in points:

- Males (68%) were found to be more susceptible to CML than females, (32%).
- The patients age ranged between 15 to 82 years.
- CML causes significant decrease in hemoglobin concentration and insignificantly reduction in PCV with mean .
- Significant changes in leukocytes differential count were observed.
- Immature WBCs and nucleated RBCs were seen in the peripheral blood of CML patients.

4.3. Recommendations:

The study recommends:

1. Further studies with a large sample size should be performed to:

a. investigate the bone marrow cells.

b. determine the phase of CML.

c. determine the tumors' markers in Sudanese CML patients.

2. Regular check up of haemostatic mechanisms in CML patients should be done.
3. The Health Ministry should provide more advanced facilities for accurate diagnosis of CML and hence treatment.

Chapter Five

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Appendices

5.1.Questionnaire

Patient No …… CBC No…….. Type of sample …… Date……...

Name ……

Age …….....

Sex ……

Laboratory investigation:

Hb.g/dl	Hb.%	TWBCs	Plts	N%	L%	M%	E%

B%	Stab%	Meta%	Myelo%	Pro%	Blast%	NRBCs%

5.2.Control Samples

Patient No …… CBC No…….. Type of sample …… Date……...

Name ……

Age …….....

Sex ……

Laboratory investigation:

Hb.g/dl	Hb.%	TWBCs	Plts	N%	L%	M%	E%	B%