

Chapter one

Introduction and Literature review

1.1Introduction

One blood cells that are characterized by its colorlessness (absence of hemoglobin) so they are referred to as white cells. Their main function is body defense and found in the peripheral blood within the range of $(4-11 \times 10^9/L)$. A secondary granules which materials that help them to fight pathogens .there are three cells which are granulocytes neutrophil , esinophil and basophile,(Harland, *et al.*,2002). Also the with blood cell (leucocytes) are divide in to two broad groups the phagocyte and the immunocyte .Granulocyte ,which include three type of cell -neutrophil, eosiphi land basophile-together with monocyte comp rice the phagocyte .Only mature phagocyte cells and lymphocytes are found in normal peripheral blood. The lymphocytes, and the precursor cell and plasma cell, which make up the immunocyte population ,The function of the phagocyte and immunocyte in protecting the body against infection is closely connected with two soluble protein systems of the body immunoglobulin and complement .These proteins , also involved in blood cell destruction in number of disease . (Hoff brand, *et al*, 2011).

1-2Blood Definition

Specialized vital connective tissue, opaque, alkaline in pH, and consists of two portions; fluid called plasma and solid which called cells. The temperature is 38C⁰, blood viscosity is 4.5-5.5; and it equals about (8%) of the body weight, (Harland, *et al.*, 2002).

1.3 Blood Function:

Blood has three main functions; 1-Transport. 2-Protective, and 3 -Regulation, (Harland, *et al.*, 2002).

1.3.1. Transport:

Transport of the following substances; -

- Gases, namely oxygen (O₂) and carbon dioxide (CO₂) between the Lungs and rest of the body.
- Nutrients from the digestive tract and storage sites to the rest of the body.
- Waste products to be detoxified or removed by the liver and kidney
- Hormones from the glands in which they are produced to their target cells
- Hormones from the glands in which they are produced to their target cells. - Heat to the skin so as to help regulate body temperature. (Salad inks, 2004).

1.3.2 Protective:

Blood has several roles in inflammation; - Leukocytes, or white blood cells, destroy invading micro organism and cancer cells. - Antibodies and other proteins which destroy pathogenic substances. - Platelet factors initiate blood clotting and help minimize blood loss (Salad inks, 2004).

1.3.3 Regulation

The PH by interacting with acids and bases. -Water balance by transferring water to tissues , body temperature

1.4. Blood Composition:

Blood composed of pale yellow fluid called plasma in which are suspended red cells (erythrocytes), white cells (leucocytes), and platelets (thrombocytes) ,(Harland, *et al.*,2002).

1.4.1. Plasma:

Forms about 55% of blood volume and contains water 90% and many solutes including proteins, minerals, irons, organic molecules, hormones, enzymes, products of digestion, and waste products for excretion, (Harland, *et al.*,2002).

1.4.2. Blood Cells:

There are three types of blood cells that are suspended in plasma which are;

1-Red blood cells(erythropoiesis)

2-Platelets(thrombocytes) and

3-White blood cells(leucocytes,(Harland, *et al.*,2002).

1.4.2.1 Red blood Cells(Erythrocytes);

RBCs are biconcave disk shaped a nucleated cells produced from bone marrow under the influence of erythropoietin hormone .RBCs contain hemoglobin which is responsible for gaseous exchange(CO₂ and O₂),RBCs express CR1 on their membrane which help in immunologic removal of tissue depress from the circulation .Normally RBCs count in peripheral blood is(4.5-5.0*10⁹/L)in adult males and (4.0-4.5)in adult females,(Harland, *et al.*,2002).

1.4.2.2 Platelets(Thrombocytes):

Thrombocytes are 2-4 um in size and a nuclear, their light blue stained cytoplasm and their progress give them a star like shape, reddish blue granules near the centre. Young thrombocytes are more spread out ;older ones look like psychotic dotes.

Thrombocytes are produced in bone marrow from a mature megakaryocytic (150-450*10⁹/L) and have an important role in prevention of blood loss by making primary

1.4.2.3 White blood Cells(Leucocytes):

One blood cells that are characterized by its colorlessness(absence of hemoglobin)so they are referred to as white cells. Their main function is body defense and found in the peripheral blood within the range of $(4-11 \times 10^9/L)$ secondary granules which materials that help them to fight pathogens .there are three cells which are granulocytes,(Harland, *et al.*,2002).

.1.4..2.3.1 Granulocytes:

They are known as granulocytes because of their cytoplasmic granulation (primary and secondary granules)which contain hydrolytic enzymes ,nitrogenoxide ,hydrogen peroxide and other materials that help them to fight pathogens. There are three cells which are granulocytes. ,(Harland, *et al.*,2002).

- Neutrophil Granulocyte
- Eosinophil Granulocyte
- Basophil Granulocytes

1.4..2.3.2 Agranulocytes:

Because of absence of cytoplasmic granulation there for they are known as a granulocyte, and include: 1- Monocyte. 2- lymphocyte, Harland, *et al.*,2002).

- Monocyte

-Lymphocytes

1.5 Blood Formation "Haemopoiesis"

1.5.1 Definition

Haem: Latin word means blood poiesis latin word means production. Is the production of the faulted elements of blood

1.5.2 Sites of haematopoiesis

The sites of haematopoiesis depend on the presence of disease and on the developmental state of the individual. Under normal conditions, all cellular components originate in the bone marrow. Some components (e.g., erythrocytes and platelets) complete their development at medullar (i.e. bone marrow) sites, whereas other components (e.g. T and B cells) complete their development at extra medullar sites, (Emmanuelli *et al.*, 1992). Fetal sites of hematopoietic: In uterus, hematopoietic proceeds as follows:

- 1- hematopoietic can be detected first in the blood islands, groups of mesenchymal cells in the yolk sac, at 3-12 weeks gestation.
- 2- The liver is active in hematopoietic from 5-6 weeks up to 6 months gestation and even as long as 2 weeks after birth.
- 3- The spleen is active at 4-8 months gestation.

4- Bone marrow becomes active at about 5 months gestation and becomes the primary site by 7 months. The bone marrow remains the principal site of hematopoietic after birth- Postnatal changes in the sites of hematopoietic:, all bone marrow cavities are hematopoietic.

5- ● The marrow cavities of peripheral bones stop producing cells.

● The marrow cavities of the axial skeleton becomes more prominent until, after 20 years of age, blood production has become limited to the vertebrae, sternum, iliac bones, skull, and proximal ends of the long bones of the extremities. ,(Emmanuell *et al.*, 1992).

1.5.2.1 Erythropoiesis

Formation of red blood cell. Red cells are produced by proliferation and differentiation of precursors whose dominant representatives in the bone marrow are the erythrocytes. Erythroblasts referred to as normoblasts when their morphological features are within normal limits. During the course of differentiation, the size of the erythroblasts progressively decreases and the character of the nucleus and cytoplasm changes the cells proceed toward the point where proliferative capacity is lost and (hemoglobin becomes the predominant protein the cytoplasm -Pro erythroblast (Pro normoblast): is the least maturation of the

morphologically identifiable members of the erythroid series. It has a diameter of 14-20p,m, and a basically round outline with minor peripheral protuberances. There are several nucleoli in the. nucleus, the chromatin in the nucleus consists of a net work of fine red purple strands -Basophilic erythroblast (Early normoblast): - Is around cell with diameter of 12-1611m, and more basophilic cytoplasm than the pro erythroblast Polychromatophilic erythroblast (intermediate normoblast): Is around cell between 12-14p.m in diameter the characteristic polychromatic appearance of the of the cytoplasm derived from the mixture of the basophilic ribonucleic acid (RNA), and a cidophilic haemoglobin. Nuclear chromatin is coarse , deeply basophil clumps Orthocluomatophilic erythroblast (Late normoblast): Constitute the next final stage of maturation of the nucleated red cell series. They are smaller than their produces , and have a diameter between 8 and 12p.m. Nucleus small and pyknotic, with a homogeneous blue-black appearance). Reticulocyte: Have the same biconcave discoid shape as mature red cells, although they have a slightly greater volume and diameter than the latter (Erythrocyte (Mature RBCs): Biconcave discoid shape, with diameter 7.2p.m. , a nuclear cell, with central pale area .(Pennington, *et al.*, 1989).A complete blood count (CBC) is a series of tests used to evaluate the composition and concentration of the cellular components of blood(Chemecky,2001).

1.5.2.2 Thrombopoiesis:

Thrombopoiesis refers to the production of platelets in the blood, because of this platelets called thrombocytes. This starts when a haemocytoblast develops receptors for the hormone thrombopoietin which is produced by the liver and kidneys. When these receptors are in place, the haemocytoblast becomes committed cell called megakaryoblast. This replicates its DNA (Baker, 1976).

1.5.2.3 Leucopoiesis

The process of production white blood cell, include;

1.5.2.3 .1 Granulopoiesis

They are known as granulocytes because of their cytoplasmic granulation (primary and secondary granules) which contain hydrolytic enzymes, nitrogen oxide, hydrogen peroxide and other materials that help them to fight pathogens. There are three cells which are granulocytes, (Harland. *et al.*, 2002). The predominant white blood cell, or leucocytes, in the circulation is mature granulocyte. The granulocyte series, such as, myeloblasts: Large cell, 15-20µm in diameter, with round to oval nucleus, no typical granules in the moderately basophilic cytoplasm. Nuclear chromatin is red-purple strands, nucleoli are two or three is usual number (Penington, *et al.*, 1989)

●Neutrophilic Granulocyte "Neutrophil

Band cells "band neutrophil"present the further development of meta myelocyte which is characterized by un segmented band shape nucleus and represent about 2% in the peripheral blood. Segmented Neutrophil ; is the final cell in the lineage started with myeloblaste characterized by segmented nucleus 10-20% have two segments,40-50% have three segments,10-20% have four segments, and 0-5% have five segments, fine blue cytoplasm granules. Neutrophils are phagocytic cell against bacteria and represent about 50-70% in peripheral blood,(Harland. *et al.*,2002).

●Eosinophilic Granulocyte (Eosinophil)

Eosinophil arise from same stem cell population as neutrophil, and mature in parallel with them, generally eosinophil are characterized by bi lobed nucleus, rough red orange granules. Normally represent about 1-6% from the peripheral WBCs count, active against parasitic infections (Harland. *et al.*,2002).

● Basophilic Granulocytes "Basophile

Like eosinophil, basophile mature in parallel with cells of neutrophil lineage the earliest stage at which they can be identified is the myelocyte stage at which large black violet stained granules are present. Basophil have bilobed

nucleus which covered by large dark violet cytoplasm granules, normally they represent about 0- 1% from the peripheral leucocyte count. Basophil like tissue mast cells are responsible for allergic and hyper sensitivity reactions,(Harland. *et al.*,2002).

1.5.2.3 .2Agranulocytes

Because of absence of cytoplasmic granulation they are known as a granulocyte, and include: monocytes and lymphocytes

●Formation of monocytes

Monocyte is a largest cell in the peripheral blood "20-40um" and has an ovoid nucleus Usually irregular and often pseudopodia like cytoplasmic process, their cytoplasm is stained light gray blue. Monocytes is the main phagocytic cell and gives arise to tissue macrophage, they represent about 2-10% of the total leucocytes count,(Harland. *et al.*,2002).

Monocyte development line is branches off at very early stage from that of granulocytic series but does not contain any distinct, specific precursor that can be surely identified .

e. Cytoplasm is abundant, and of apple grey to blue tinge. It contains some small neutrophilic or basophilic granules. Monocytes are motile cells and are thus

capable of migrating into the blood passing through bone marrow sinusoids ((Penington,*et al.*, 1989).

● **Lymphocytes**

Lymphocytes are produced everywhere, particularly in the lymph node, bone marrow, and lymphatic islands of intestinal mucosa under the influence of thymus gland "T-lymphocytes 80%" or bone marrow "B-lymphocyte 20%" a few fraction of lymphocytes are natural killer cells (NK). Morphological lymphocytes are classified into small and large lymphocyte. Lymphocyte represent about (20-40%) of peripheral WBCs count, and active against viral infections, and they are the most important immune cells,(Harland. *et al.*,2002).

Production of the lymphocyte, the lymphoid series -Lymphoblast's; are slightly smaller than the myeloblasts which they resemble, except that the ratio of the diameter of the nucleus to that of the cell tends to be greater, and the number of nucleoli to be fewer than in myeloblasts(Penington,*et al.*, 1989).

●The large lymphocyte; Is between 12 and 14pm in diameter, the nucleus is round or indented, chromatin more clumped than in the lymphoblast. Cytoplasm abundant, and usually pale blue. (Penington,*et al.*, 1989)

●The small lymph('yet; Is between 9 and 12 μm in diameter, and smaller than Segmented granulocytes, cytoplasm is scanty(Penington,et al., 1989) .

1.5.3.Haemopoiesisgrowthfactors

Include s; GM-CSF (Granulocytes. monocyte, colony, stimulating, factor), G-CSF (Granulocytes, colony, stimulating, Factor-I), IL-3 (Inter, leukine-3), IL-5 (Inter, leukine-5). Erythropoietin and thrombopoietin (Hoff brand, et al., 2006).

1.6. disorders of leucocytes function

Disorders of neutrophil function can be classified into congenital versus acquired disorders. They can further be classified into disorders of chemo taxis, decreased phagocytosis due to impaired opsonization, and defect in microbial killing. Some of the inherited disorders are associated with morphologic abnormalities in neutrophils, with or without associated abnormalities in .(neutrophil number. .(William Kern and Parker, 2002

Congenital Disorders of leucocytes Function .1.6.1

Chronic NADPH oxidase; Majority X-linked Recurrent●
 .infectionwith catalase-positive organisms
 Granulomatous cytochrome (~75%); remainder
 inflammation, infections disease (CGD) subunit
 .autosomal recessive

Myeloperoxidase. Autosomal recessive May have ●
disseminated Candida or other fungal infections,
particularly in

MPO) deficiency diabetics; majority of patients have no)
.increase in infections

Leukocyte adhesion _2 integrin chain autosomal •
recessive Recurrent infections (gingivitis, per identities)
without neutrophil. deficiency type 1 (CD18)
response; persistent neutrophilia

- Chédiak-Higashi; Granule fusion autosomal
recessive Partial oculous tenuous albinism; large
lysosomal granules in PMNs, monocots, syndrome
melanocytes and other cells; increased
susceptibility to pyogenic infections ;may
terminate as lymphoma-like accelerated phase
Specific grannules Absence of secondary
Autosomal recessive Recurrent skin and sinus
infections, predominantly with staphylococci
deficiency granules.(William Kern and Parker,
2002).

Alder-Reilly anomaly Incomplete degrade, autosomal •
recessive Large purple granules in PMNs,
lymphocytes, and monocots; normal neutrophil
function of mucopoly- function, with no increased
susceptibility to infection; associated with
saccharides mucopolysaccharidoses (Hunter's and
(Hurler's syndromes

May-higlen Autosomal dominant Large pale blue •
granules in PMNs resembling Döhle's bodies;
thrombocyto peniaanomaly with giant platelets;
.defects in platelet function; no increase in infections

Pegler hu't •

Autosomal dominant Majority of PMNs have bi lobed
.nuclei ("pince-nez" cells), with a few tri lobed

Anomaly nuclei; coarse nuclear chromatin; no •
increase in susceptibility to infection lymphadenitis,
.cutaneous infections, and impetigo

1.6.2. Disorders with Abnormalities in leucocytes numbers

Neutrophilia •

Neutrophilia is defined as an absolute neutrophil •
,count greater than ~7,000/_L in the adult instances

Causes of neurophilia •

Infections •

Inflammatory disorders: rheumatoid arthritis, other •
autoimmune diseases, gout, others

Acute stress or physical exertion, seizures •

Acute hemorrhage •

Haemolysis: acute or chronic •

Metabolic disorders: diabetic ketoacidosis •

- .Hodgkin's disease
 - .Non-hematologic malignancies
 - Medications: lithium, corticosteroids, epinephrine, .hematopoietic growth factors
- Chronic idiopathic neutrophilia. .(William Kern and Parker, 2002).

Neutropenia

●Neutropenia is defined as an absolute neutrophil count (segmented + bandneutrophils) less than ~1,500/_L for Caucasians and less than ~1,200/_Lfor African Americans. The term a granulocytosis is sometimes used to indicates ever neutropenia. .(William Kern and Parker, 2002).

(Calculation of Absolute Neutrophil Count (ANC

$$ANC = WBC _ (Sags [\%] + Bands[\%]) _ 0.01$$

Eosinophilia

Eosinophils are typically reported as percent of leukocytes, with the normal range being approximately 0 to 7%.Any eosinophil count >7% is often considered osinophilia. However, eosinophilia is better defined based on the absolute number of eosinophils in the blood rather than as a percent of white cells. Therefore, eosinophilia is defined as >700 eosinophils per micro liter(>0.7 _ 10³/_L or >0.7 _ 10⁹/L). Eosinophilia is

sometimes divided into mild ($0.7-1.5 \times 10^3/\text{L}$), moderate ($1.5-5.0 \times 10^3/\text{L}$), and marked ($>5.0 \times 10^3/\text{L}$) eosinophilia

(Causes of Eosinophilia(NAACP

N—Neoplasm's

A—Allergies (including drug reactions) and asthma

A—Addison's disease

C—Collagen vascular diseases

P—Parasitic (and other) infections

-Basophilia

Basophilia is defined as >150 basophiles/ L ($>0.15 \times 10^9/\text{L}$)

Causes of Basophilia

(Chronic myelogenous leukemia (CML) •

Other chronic myeloproliferative disorders: •
polycythemia vera, essential thrombocythemia,
myeloid metaplasia (idiopathic
(myelofibrosis

Infections: chronic sinusitis, vermicelli, smallpox •

Ulcerative colitis •

Lung carcinoma •

- Miscellaneous: hypothyroidism, iron deficiency, some chronic hemolytic anemia's ,nephritic syndromeould be identified and treated appropriately .(William Kern and Parker, 2002).

Monocytosis

Monocytosis is defined as $>1,000$ monocots/ μ L (>1.0 $\times 10^9$ /L

• Causes of Monocytosis

- Infections: viruses, tuberculosis, infective •
endocarditic, syphilis, brucellosis, parasites
malaria, trypanosomiasis), rickettsiae (Rocky Mountain)
(spotted fever
- Acute and chronic leukemia's: AML, CML, chronic •
(myelomonocytic leukemia (CMML
- Other hematologic neoplasm's: lymphomas, •
Hodgkin's disease
- Carcinomas: breast, ovary, prostate •
- Granulomatous diseases: sarcoidosis •
Inflammatory bowel disease •
- Collagen vascular diseases most common cause of •
.monocytosis

Lymphocytosis •

Lymphocytosis is defined as a lymphocyte count $>4,000/\mu\text{L}$ ($>4 \times 10^9/\text{L}$) for adults and $>9,000/\mu\text{L}$ ($>9 \times 10^9/\text{L}$) for children. It is important to distinguish between absolute lymphocytosis, which is an increase in the total lymphocyte count, and relative lymphocytosis, which is an increase in the lymphocyte percent due to neutropenia, without an increase in the absolute lymphocyte majority of lymphocytes ($\sim 85\%$) in the peripheral blood are T cells, with T helper (CD4^+) generally about twice as common as T cytotoxic/suppressor (CD8^+) cells. The majority of reactive lymphocytoses also consist predominantly of T cells. In contrast, the majority of lymphocytic leukemias consist predominantly of B cells. (William Kern and Parker, 2002).

Causes •

There are many possible causes of lymphocytosis. In children and young adults, lymphocytosis is almost always reactive, due to infections.

The main concern is to exclude acute lymphoblastic leukemia. In

- older adults, sustained lymphocytosis is very suggestive of chronic lymphocytic leukemia (CLL), although reactive lymphocytosis can occur at all ages. (William Kern and Parker, 2002).

Viral infections: These infections are a very common cause of lymphocytosis, particularly in children. Virtually

any virus can be associated with lymphocytosis; however; three viruses are particularly prone to cause marked lymphocytosis: Epstein-Barr virus (EBV), which causes infectious mononucleosis; cytomegalovirus (CMV), which causes most cases of “Mono spot”-negative infectious mono; and viral hepatitis. The lymphocytes associated with mononucleosis are typically large, with abundant pale blue cytoplasm, enlarged nuclei with fine nuclear chromatin, and prominent nucleoli. They are usually called atypical lymphocytes, but I prefer the term “reactive.” The reactive lymphocytes often appear to “hug” surrounding erythrocytes. Interestingly, EBV infects B lymphocytes; however, the large lymphocytes seen in the blood are T cells, presumably reacting to the infected B cells. (William Kern and Parker, 2002

Causes of Lymphocytosis

- Viral infections, particularly EBV, CMV, viral hepatitis
- (Bordetella pertussis (whooping cough
- Toxoplasmosis
- Brucellosis
- Acute infectious lymphocytosis
- Tertiary or congenital syphilis
- Drug hypersensitivity reactions

Endocrine disorders: thyrotoxicosis, Addison's •
.disease, hypopituitarism

.Persistent polyclonal B-cell lymphocytosis •

- B-cell chronic lymphocytic leukemia and other lymphocytic leukemiastecount. Explained abnormalities.(William Kern and Parker, 2002).

- **1.7 Insulin**

Insulin is a protein produced by the cells of the islets of Langerhans of the pancreas. Insulin is the first protein hormone to be sequenced, the first substance to be measured by radio immunoassay (RIA) and the first compound produced by recombinant DNA technology for practical use. It is an anabolic hormone that stimulate the up- take of glucose into fat and muscle:

- promotes the conversion of glucose to glycogen or fat for storage

- Inhibits glucose production by the livers

- Stimulates protein synthesis and inhibit protein breakdown.

Human insulin (molecular mass 6000D) consists of 51 amino acids in two chains (A and B) joined by two disulfides bridges with a third disulfide bridge within the A chain.

Insulin from most animals is similar immunologically and biologically to human insulin and in the past all insulin

dependent patients were treated with insulin purified from beef or pig pancreas. Virtually all patients are now treated with recombinant human insulin. (Burris and Ashwood, 2001).

Pre pro insulin, a protein of about 100 amino acids, is not detectable in the circulation under normal conditions because it is enzymatically cleaved and converted to pro insulin. Pro insulin is stored in secretory granules in the Golgi complex of the β -cells, where pro hemolytic cleavage to insulin and connecting peptide (C-peptide) occurs. This post translational processing is catalyzed by two Ca-regulated endow peptidases, namely pro hormone convertase 1 and 2 (PC1 and PC2). The split pro insulin intermediates, split 32,33 pro insulin and split 65,66proinsulin, are further hydrolyzed to insulin and C-peptide. At the cells membrane the insulin and C-peptide are released into the portal circulation in equimolar amounts. In addition, small amount of pro insulin and inter immediate cleavages fauns enter the circulation(Burris and Ashwood, 2001). Pro insulin, which has relatively low biological activity (approximately 10% of insulin potency), is the major storage form of insulin. No lineally only small amounts (about 3% of the amount of insulin, on a molar basis) of Pro insulin enter the circulation. Because the hepatic clearance of Pro insulin is only 25% of insulin clearance, the half-life of pro insulin is twofold to three fold longer and concentrations

in the fasting state are approximate 10% to 15% of insulin concentrations. (Burris and Ash wood, 2001) .

C-peptide is devoid of biological activity but appears necessary to ensure the correct structure of insulin. Although insulin and C - peptide are secreted into the portal circulation in equimolar amounts, fasting concentrations of C- peptide are fivefold to tenfold higher than those of insulin due to the longer half- life of C- peptide (about 35minutes). The liver dose not extract C- peptide, which is removed from the circulation by the kidneys and degraded, with a fraction excreted unchanged in the urine(Burt
is and Ash wood, 2001).

1.7.1Diabetes Mellitus (D.M)

Diabetes mellitus is a group of metabolic disorders of metabolism in which glucose is underutilized producing hyperglycemia. Some individuals may experience acute life threatening hyperglycemic episodes such as keto acidosis or hyposmolar coma. (Burris and Ash wood, 2001). It is a syndrome of chronic hyperglycemia due to relative insulin deficiency or resistance or both. (Parveen Kumar *and* Michael,(2006) .Diabetes is usually irreversible and although patients have reasonably normal life style ,is late complications result in reduce

life expectancy and major health costs, (David ., *et al* 2005). Diabetes is widely recognized as one of the leading causes of death ,The rapid increase in diabetes parallels the increase in obesity and overweight, recent information indicates that 5.5%in northern of Sudan and 8.6% in Khartoum state have diabetes and the number is expected to rise (Elbagir ,2006).

1.7.2 Classification of Diabetes Mellitus

1) Type 1 diabetes mellitus

Type1 was formerly known as insulin dependent diabetes mellitus (IDDM), type I or juvenile onset diabetes. Approximately 5% to 10% of all individuals with diabetes mellitus have type I diabetes. Symptoms usually present acutely, diabetic individuals have insulinopenia (a deficiency of insulin) because of loss of pancreatic islet cells and depend on insulin to sustain life and prevent ketosis. Most individuals have antibodies that identify an autoimmune process, some have no evidence of autoimmunity and are classified as type I idiopathic. The peak incidence of this disease is in childhood and adolescence. Approximately 75% acquire the disease before 30 years of age, but the onset in the remaining percentage of individuals may occurs at any age((Burris and Ash wood, 2001)

2) Type2 diabetes mellitus

Type2 diabetes mellitus is characterized by hyperglycemia as result of an individual's resistance to insulin with an insulin secretory defect .This resistance result in relative , not an absolute insulin deficiency(Bishop,2010).

Type2 formerly known as non- insulin dependent diabetes mellitus (NIDDM). Type II or adult onset diabetes- comprise approximately 90% of all individuals with diabetes. Individuals have minimal symptoms, are not prone to ketosis, and do not depend on insulin to prevent ketonuria. Insulin concentration may be normal, decrease, or increased and most people with this form of diabetes have impaired insulin action. Obesity is commonly associated with the condition, and weight loss alone frequently ameliorates the hyperglycemia. However many individuals with type2 diabetes may require dietary manipulation, an oral hypoglycemic agent or insulin to control hyperglycemia. The disease usually develops after 40 years of age, but type2 diabetes may occur in young people particularly those who are obese(Burris and Ash wood, 2001)

3) Impaired glucose tolerance

It is diagnosed in people who have fasting blood glucose concentrations less than those required for diagnosis of diabetes mellitus but have plasma glucose response during GTT between normal and diabetic. A GTT is required to assign an individual to this class. Micro

vascular disease is quite rare in this group and individuals usually do not experience the renal or retinal complications of diabetes. (Burris and Ash wood, 2001)

4) Gestational diabetes mellitus

GDM is defined as glucose intolerance with onset or first recognition during pregnancy, that is diabetic women who become pregnant are not included in this category. Estimates of the frequency of abnormal glucose tolerance during pregnancy range from 1% to 20% but the true incidence of GDM is probably 3% to 5%. Women with GDM are at increased risk for the subsequent development of diabetes mellitus ((Burris and Ash wood, 2001)

5) Insulin resistance

Insulin resistance is defined as a decreased biological response to normal concentrations of circulating insulin and is found in both obese, non diabetic individuals and those with type 2 diabetes. A broad spectrum of insulin resistance exists ranging from normal blood glucose concentrations (although marked elevation in endogenous insulin) to hyperglycemia despite large doses of exogenous insulin. Several rare clinical syndromes are associated with insulin resistance. The prototype is the type A insulin resistance syndrome, which is characterized by hyper insulinemia, acanthosis nigricans and ovarian hyperandrogenism ((Burris and Ash wood, 2001)

1.7.3 Causes of Diabetes Mellitus

1\ Causes of type 1 D.M

- I cells autoimmunity due to circulating anti bodies such as islet cell cytoplasmic anti bodies, insulin auto antibodies and antibodies to glutei acid de carboxylase .
- Genetic factor lead to susceptibility to type 1DM.
- Environmental factors such as viruses and chemicals.

2\ Causes of type 2 DM

- Genetic factor (diabetogenes).
- Environmental factors such as diet and exercises.
- Loss of β -cells function due to increased demand of insulin

(Burris and Ash wood, 2001)

1.7.4 Diagnosis of Diabetes Mellitus

Diabetes is easy to diagnose when overt symptoms are present and a glucose tolerance test (GTT) is not necessary for most clinical purpose. The oral glucose tolerance test (GTT) has however, allowed more detailed epidemiological characterization based on the existence of separate glucose thresholds for macro vascular and micro vascular disease. These correspond with the levels for the diagnosis of impaired glucose tolerance (IGT) and diabetes as specified by the. (Praveen Kumar and Michael, 2006)

WHO criteria are:

- Fasting plasma glucose >7.0 mmol/l (126mg/dl)
- Random plasma glucose >11.1 mmol/l (200mg/dl)
- One abnormal laboratory value is diagnostic in symptomatic Individuals; two values are needed in asymptomatic people. The glucose tolerance test is only required for borderline cases and for diagnosis of gestational diabetes
- The GGT-W1-10 criteria Normal IGT DM Fasting less than (7.0)mol/l less than (7.0)mol/l more than (7.0)mol/l 2h after glucose less than (7.8)mol/l between (7.8-11.0)mol/l (11.0)mol/l or more (WHO,1999).

1.8 WBC associated with insulin sensitivity

One possible explanation is that both a higher WBC and insulin resistance reflect an underlying activation of the immune system. It was shown, for instance, that interleukin-6 (IL-6), a potent white blood cell differentiation factor that is produced mostly in adipose tissue is associated with insulin resistance. (Fernandez Real.*etal.*,2001 d).Therefore, it could be hypothesized that IL-6 may be a factor that not only increases WBC but also causes insulin resistance. This notion is also supported by an observation that a single nucleotide polymorphism in the IL-6 gene was shown to be associated with an increase wbc's and lower insulin

sensitivity.(Fernandez.,*et.*, *al* 2000). Interestingly, it has been shown that WBC and other markers of inflammation aggregate in families, which suggests that genetic factors may be involved in the activation of the immune system ([partly](#) ,*et al* ., 1995). However, because relatives share not only genetic determinants but also environmental factors, such as exposure to infection, it is not possible to determine whether familiar associations are genetic or environmental. Because cytokines, such as IL-6, are produced by activated white blood cells, it is also possible that an activation of the immune system, caused by inflammation, could increase WBC and therefore cytokine production(Pickup,*et al* .,1997), which may decrease insulin sensitivity. Hormones are another possible link between WBC and insulin sensitivity. A variety of hormones have receptors on the surface of white blood cells and have been shown to play a role in their production and maturation (Festal ,*et al.*,2000).Some of them, such as insulin, cortisol, and sex hormones, are also associated with insulin resistance The role of cortisol and sex hormones as a possible link between WBC and insulin resistance cannot be resolved in this study. Insulin is an another possible link; however, we observed no difference in fasting plasma insulin concentrations between pro aggressor and non pro aggressors at baseline, nor a relationship between

baseline WBC and follow-up insulin concentrations.
([Freeman](#), *et al.*, 2001).

1.9Rational

diabetes mellitus is one of major health the problems in Sudan resulting in 10% of olla hospital admission and mortality , according to the WHO more than 150 million people worldwide a suffer from diabetes mellitus it is increasing rapidly ,and it is estimated by years(2025) this number will be double . The exact number of people

with diabetes mellitus in Sudan is not known ,a small population based study in 1993 of sample of 1284 adult men ,showed prevalence of 3.4% type diabetes mellitus. Diabetes mellitus in Sudan is associated with high rate of ,coronary, heart disease ,blindness and renal failure and incidence of diabetes mellitus is progressively increasing Diabetes mellitus occurs through the world ,but is more common epically(type2)in the developed countries. diabetes mellitus is in top 10 and perhaps top 5. it is the most significant disease in develop world.

Many studies reported result for high level of total white blood cell counts associated with type 2 diabetes mellitus in different countries .this study aimed to find out if there is a significant change in the total white blood cell and absolute values of white blood cell in Sudanese patients with type 2 diabetes mellitus .

1.10Objective

2.10.1 -General objective

-To study the total counts and absolute white blood cell in Sudanese patients with type 2 diabetes mellitus.

2.10.2 -Specific objective

-To determine neutrophil counts, lymphocyte counts and mixed cell count in patients with type 2 diabetes mellitus.

-To study the correlation between the duration of type 2 diabetes mellitus and total counts and absolute values of white blood cell.

-To study the effects of age and gender of diabetic patients on total counts and absolute values of white blood cell.

Chapter Two

Materials and Methods

2.1 study Design

This is analytical case control study to determine total counts and absolute values of white blood cells in diabetic patients carried out in jabber Abu Eliza center during the period from march to august 2015.

.2.2 study area

The study was conducted at jabber Abu Eliza center during the period from March to August/ 2015.

2.3 study population

Hundred blood sample were collected 80 blood sample from . patients with type 2 diabetes mellitus and 20 blood sample from healthy non diabetic individual.

2.4 study duration

The study was carried out at the period from march\2015 to august \2015)

2.5 Inclusion criteria

patients diagnosed with diabetes mellitus type 2

2.6 Exclusion criteria

Patient with known cases of other cause of increase total counts such as diabetic septic food were excluded from the study

2.7 sample collection

Five ml of venous blood was collected from diabetic patients and non diabetic individual, by syringe in to EDTA containers .EDTA blood samples were analyzed by automated hematological analyzer (sysmex).

.2.7-1principle of System

System instruments, manufactured by TOA. Medical electronics company , included a full line of hematology analyzers from the smallest. Which performed complete blood count, and five parts of differential (white blood cells red blood cells hemoglobin packed cell volume , and platelets count) are considered to be measured directly three hydraulic sub systems are used to determine the hologram : the white blood cells channel , red cell, Platelets channel , and separate hemoglobin channel. In white cells transducer chambers, the diluted white blood cells and red cells samples are separated through the different apertures and are Counted by using the electronic resistance (impedance)) detection method for counting and sizing cells. Two unique features enhance the impedance technology. In the red blood cells, Platelets channel, a sheeted stream with hydrodynamic focusing is used to direct the cells in single file through the aperture, there was reducing coincident passage and pulse height irregularities, and in both the white blood cells and red blood cells, platelets channels (floating thresholds) are used to

discriminate each cell population. As the cells pass through the apertures the signals are transmitted in sequence to the analog circuit and then to the particle size distribution analysis circuits for conversion to cumulative cell size distribution data. Particle size distribution curves are constructed, and the optimal position of the auto discrimination level is then set by the micro processor for each cells population for example, the lower platelets threshold may be set between 26 fl and the upper threshold anywhere from 12-30 fl based on the particle size distribution, likewise, the red blood cells lower and upper thresholds may be set between 25-75 fl, and 2000-250 fl, respectively. This floating threshold circuitry allows for discrimination of cells population on sample. By sample basis, the cells count include the pulses between the lower and upper auto discrimination levels , with dilution ratio , volume counted , and coincidence error accounted for in the final computer generated counts. In the red blood cells channel , the floating discriminator is particularly useful in separating platelets from small red blood cells. The hematocrit is also determined from red blood cells/platelets channel, based on the principle that pulse height of the red blood cells is proportional to cell volume. The hematocrit is then the cumulative pulse height and is considered true relative percentage volume of red cell (erythrocyte). In the hemoglobin flow cell, the concentration of cyanine the mol bin is

measure as absorbance at 540 nm for the hemoglobin concentration. The following indices are calculated in the microprocessor using the directly measured or derived parameters: Mean cell volume (MCV) , mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) red cell width standard deviation (RDW.SD) RDW. CV, MPV, platelet distribution width (PDW), and platelet large cell ratio(P.LCR). The red blood cell ratio(P.LCR).the RDW.SD is the is red blood cell aright distribution width measured at 20% of the height of the red blood cell curve , reported in flit liters with reference interval of 37-54 flit.RDW.CV is the red blood cell distribution width reported as a coefficient of variation. (Ropak F. 1995)

1.8. Data collection

Data was collected by personal interview questionnaire to the diabetic patient including gender, age and the duration of D.M data were analyzed by (spss version 11.5) qualitative data was represented as frequency and percentage ; quantities data was represented as mean SD mean of quantities variable were compared using independent 2. sample T-test - between age , duration of D.M with white blood parameters was tested person correlation .

2.8.1 Data analysis

The data obtained were categorized in to different group and analyzed by statistical spas computer program.

2.9 Ethical consideration

Informed consent was obtained from each participant prior to involvement in the study.

Chapter Three

Results

Eighty patients with type 2 diabetes mellitus attended Jabber Abu Eliza center ,were enrolled in study as test group and 20 apparently health adult people as control group. performed total white blood cell and absolute values of white cell

1.basic data of study subject

all diabetic participant were found to be with type2 diabetes mellitus. -(**Table 3-1**). Distribution of study population according to Gender

The majority of the case were male, frequency (45),percentage (56.2%) while the female frequency(35,)percentage (43.8%), the control, the male frequency (11),percentage (55%) while the female frequency(9)percentage (45%).

The mean of age of study subject is 51.86 ,SD 10..97 the minimum age is 28years and the maximum age is 77years . (**Table3-2**).

-It is presented in.(**Table3-3**).The effect of type2 diabetes mellitus on total count and absolute values of white blood cell ,which shows that there was significant increase in the mean of total counts and absolute values of white blood cell in case group than control group

-Effect of the gender on total counts and absolute values of white blood cell. (**Table 3-4**).shows that ,the gender did not have significant effect on total counts and absolute values of white blood cell among type2 diabetic subject.

It is presented that the correlation between total counts, absolute values of white blood cell and age .showed there was no statistically significant correlation between them. (**Table3-5**.)

. (**Table3-6**.).The correlation between total counts, absolute values of white blood cell and duration of DM. ,shows that there is statistically significant

correlation between total counts, absolute values of white blood cell and duration of DM.

-(Table 3-1) Distribution of study population according to Gender

Total	Control	Patients	Gender
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	Percentage(%)	Frequency	Percentage(%)	Frequency	
56	55%	11	56.2%	45	Male
44	45%	9	43.8%	35	Female

-(Table3-2) the mean of age, minimum age and .maximum

Maximum	Minimum	SD	Mean	
77	28	10.974	51.86	Age

-(Table 3-3) Effect of type2 diabetes mellitus on total count and absolute values of white blood cell.

<i>p. value</i>	SD	Mean	Populati on	Parameter
0.00	3.483	9.720	Case	Total count*10 ⁹ /L
	0.797	4.510	Control	
0.00	3.577	6.685	Case	Neutrophil count *10 ⁹ /L
	0.490	2.265	Control	
0.038	0.872	2.221	Case	Lymphocyte count *10 ⁹ /L
	0.397	1.800	Control	
0.00	0.414	0.818	Case	Mixed cell count*10 ⁹ /L
	0.195	0.460	Control	

(p.value ≤ 0.05)

-(Table 3-4).

Effect of the gender on total count and absolute values of white blood cell.

<i>p. value</i>	SD	Mean	Population	Parameters
0.337	3.892	9.000	Male	Total counts
	3.603	8.268	Female	*10 ⁹ /L
0.254	3.766	6.173	Male	Neutrophil counts*10
	3.511	5.327	Female	⁹ /L
0.209	0.766	2.044	Male	Lymphocyte counts*10
	0.869	2.254	Female	⁹ /L
0.223	0.456	0.791	Male	Mixed cell counts*10
	0.329	0.695	Female	⁹ /L

(p.value ≥ 0.05)

(Table 3-5).There was no statistically significant correlation between patients age and each of total counts and absolute values of white blood cell and age

Parameters	Pearson Correlation	<i>p.value</i>
Total counts*10 ⁹ /L	.022	0.832
Neutrophil *10 ⁹ /L	.042	0.677
Lymphocyte	-.165	0.101
Mixed cell *10 ⁹ /L	.119	0.240

(*p.value* ≥ 0.05)

(Table 3-6).-There was statistically significant correlation between the duration of type 2 diabetes mellitus and each of total count and absolute values of white blood cell

Parameters	Pearson Correlation	<i>p.values</i>
Total counts*10 ⁹ /L	0.600	0.000
Neutrophil*10 ⁹ /L	0.545	0.000
Lymphocyte*1 0 ⁹ /L	0.093	0.055
Mixed cell*10 ⁹ /L	0.432	0.000

(p.value ≤ 05)

Chapter Four

DISCUSSION

Diabetes mellitus are group of metabolic disease in which a person has high blood glucose because the body does not produce enough insulin or because cell do not respond to insulin that produced (lawernce.et al.,2008).This case control study was conducted at jabber Abu Eliza specialized center during the period from March to August. It aimed to study total counts and absolute values of white blood cell in type 2 diabetes mellitus patients .The result of the present study showed that were all the total counts and

absolute values of white blood cell were increase in patients with type 2 diabetes mellitus than non diabetic control group . The(mean \pm SD) in case group 9.72 ± 3.48 in control group 4.51 ± 0.79 for total counts, 6.68 ± 3.57 and 2.26 ± 0.49 for neutrophil, 2.22 ± 0.87 and 1.80 ± 0.39 for lymphocyte, 0.81 ± 0.41 and 0.46 ± 0.19 for mixed cell. ($p.value \leq 0.05$) . These findings are consistent with the hypothesis that a chronic activation of the immune system may play a role in the pathogenesis of type 2 diabetes. Activation of the immune system may be detected by an increase in a number of markers, including white blood cell count and cytokine and plasminogen activator inhibitor-1 (PAI-1) concentrations. This is result agree with that obtained by ((Schmidt,et al.,1999),in Iran during(2007 - 2008),who stated That the average leukocyte count in these patients was $7594 \pm 1965/\text{mm}^3$. Leukocyte count was significantly a high with diabetes mellitus patients, also agree with study ,done in Pima Indian obtained by(wryer, et al.,1995),who stated ,that there was significant association bet ween the total counts and type2 diabetes mellitus ,also our study obtained by(Festal ,et al.,2000), in(2004) on Chinese diabetic patients in Hong Kong, it was demonstrated that leukocyte counts is high. Also our study related to that there was no statistically significant variation in total counts and absolute values white blood cell according to the gender the(mean \pm SD) in male group 9.00 ± 3.89 in female group 8.26 ± 3.60 for total counts, 6.17 ± 3.76 and 5.32

± 3.51 for neutrophil, 2.04 ± 0.76 and 2.25 ± 0.86 for lymphocyte, 0.79 ± 0.45 and 0.69 ± 0.32 for mixed cell. ($p.value \geq 0.05$) .Also our study indicate that there was no statistically significant correlation between total counts and, absolute values of white blood cell and age of study population,($p.value \geq 0.05$) .when the total counts and absolute values analysis within duration of diabetes mellitus ,the result showed statistically significant correlation between them,($p.value \leq 0.05$) .

4.2 CONCLUSION

- In conclusions .total counts , neutrophil counts ,lymphocyte counts and mixed counts cell were significantly increase in patients with type 2 diabetes mellitus.
- There was no difference in total counts and absolute values of white blood cell according to gender
- Total counts and absolute values of white blood cell were significantly correlated with duration of type 2 diabetes mellitus

-Total counts and absolute values of white blood cell were in significantly correlated with age of diabetic patients

4.3 Recommendations

1-Regular estimation of total counts and absolute values of White blood cell in patients with type2 diabetes mellitus.

2-Health program should be implemented to explain the complications associated with type2 diabetes mellitus.

3-More studies are needed to determine the incidence of diabetes mellitus in Sudan and to clarify the effect of hyperglycemia on total counts and absolute values of white blood cell .

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Appendix

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Total Counts and absolute values of white blood cell in diabetes mellitus type2

Questionnaire No ()

Name:

Age:

Gender: male () Female ()

Duration of D.M years

Investigation:

-Total count

- Absolute values of white blood cell

- (N)..... (M)

(L