بسم الله الرحمن الرحيم

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The Effects of Diabetes Milletus Type-II on the Complete Blood Count among Sudanese patients- Khartoum State

تأثير داء السكري- النوع الثاني على تعداد الدم الكلي في المرضى السودانيين - ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of the degree of M.Sc. in Medical Laboratory Science (Hematology and Immunohematology)

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الآيـــــة

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ: ﴿ ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلْحَىُّ ٱلْقَيُّومُ ۚ لَا تَأْخُذُهُۥ سِنَةٌ وَلَا نَوْمٌ ۚ لَّهُۥ مَا فِى ٱلسَّمَٰوَٰتِ وَمَا فِى ٱلْأَرْضِ ۗ مَن ذَا ٱلَّذِى يَشْفَعُ عِندَهُۥٓ إِلَّا بِإِذْنِهِۦ ۚ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ ۖ وَلَا يُحِيطُونَ بِشَىْءٍ مِّنْ عِلْمِهِۦٓ إِلَّا بِمَا شَآءَ ۚ وَسِعَ كُرْسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلْأَرْضَ ۖ وَلَا يَـُٔودُهُۥ حِفْظُهُمَا ۚ وَهُوَ ٱلْعَلِىُّ ٱلْعَظِيمُ ﴿٢٥٥﴾ ﴾

صدق الله العظيم

(سورة البقرة الآية ٢٥٥)

Dedication

To my, husband

To my, family

To everyone who supported me,

I dedicate this work

Acknowledgement

Thanks firstly and finally to Alla Almighty for blessing an giving me the power to complete this research.

I would like to express my deepest appreciation to my supervisor Dr. Mansour Mohammed Mansour for his patience and advices.

To a dearest soul, my backbone. Who gave me wings and made me fly. Dr. Mohammed Haj Ali.

Extended appreciation to my family and friends for their support.

Abstract

Diabetes mellitus is currently emerging as an important health problem in Sudan, especially in urban areas; which also affect the blood cells parameters in different ways in which may play a role in development of complications in diabetic patients.

This was a cross sectional study done in ALshaheeda Nada Health Center in Al Haj Yousif neighborhood in the period between February 2017 and March 2017, aimed to study the CBC parameters in patients with diabetes mellitus type II, designed and to determine the effect of DM type II on CBC parameters.

One hundred fifty subjects were recruited for this study, 100 patients with DM type II patients and 50 healthy volunteers as a control group, blood samples were collected from all participants in EDTA vacutainers tubes. Patients' data were collected by structured interview questionnaire and analyzedby statistical package for social sciences (SPSS), version 16. Complete blood count parameters were measured by automated hematologyanalyzer (Sysmex XE_2100).

There was statistically significant decrease in hemoglobin concentration, MCV, MCH and MCHC (*P.value* =0.00, 0.02, 0.01, 0.00 respectively) and no statistically significantchange in RBCs and HCT (*P.value* = 0.34 and 0.34 respectively) in DM type II patients when compared with healthy controls.

There was statistically significant increased in MXD count (*P. value* =0.04) with no statistically significant difference in platelets, WBCs, lymphocytes and neutrophil counts (*P. value* = 0.57, 0.09, 0.99, 0.51 respectively) when compared with healthy control.

This study conclude that CBC parameters HB, MCV, MCH, MCHC and Mixed count were significantly lower among diabetic subjects compared to apparently healthy controls and no significant difference on the other parameters namely RBC, WBC, PLT and HCT.

مستخلص الدراسة

يعتبر داء السكري من أكثر المشكلات الصحية ظهورا في السودان، وخاصة في المناطق الطرفية. يؤثر داء السكري على مؤشرات خلايا الدم والذي بدوره يلعب دوراً هاماً في تطور مضاعفات مرضى السكري.

هذه الدراسة المقطعية التي تمت في مركز الشهيدة ندى الصحي- منطقة الحاج يوسف في الفترة من فبراير 2017 وحتى مارس 2017 ، هدفت لدراسة تعداد الم الكلي في مرضى السكري من النوع الثاني ودراسة تأثير السكري من النوع الثاني على تعداد الدم الكلي.

شملت الدراسة 150 فرد، 100 منهم مصابين بمرض السكري من النوع الثاني و 50 منهم أصحاء كمجموعة ضابطة، جمعت عينات الدم في وعاء EDTA.

اخذت بيانات المرضى عن طريق استبيان منظم وحللت عن طريق استخدام برنامج الحزمةالاحصائية للعلوم الاجتماعية (16.0).

قيست تعداد الدم الكامل بواسطة جهاز (Sysmex XE_2100).

أظهرت الدراسات نقصان ذو دلالة إحصائية في متوسط تركيز الهيموغلوبين (Hb) ومتوسط حجم الكرية (MCV) ومتوسط هيموغلوبين الكرية (MCH) و متوسط لتركيز الهيموغلوبين في الكرية (MCHC) وقيمة P المطلقة = (0.00, 0.02, 0.007, 0.00 تباعاً)، ولا يوجد فرق ذو دلالة إحصائية في تعداد خلايا الدم الحمراء (RBCs) و حجم الكريات المكدسة (PCV) وقيمة Pالمطلقة= (0.34 و 0.34 تباعاً) في مرضى السكري من النوع الثاني بالمقارنة المجموعة الضابطة.

كما أظهرت النتائج زيادة ذات دلالة إحصائية في تعداد الخلايا المختلطة (MXD) وقيمة P المطلقة =0.04) ، و أظهرت النتائج لا فرق ذو دلالة إحصائية في التعداد لكل من الصفائح الدموية (PLTs) ،خلايا الدم البيضاء (WBCs) ، الخلايا اللمفية (LYM) و الخلايا المتعادلة (NET) وقيمة P المطلقة = (0.57،0.09، 0.99 ،0.51 تباعاً) ، بالمقارنة مع المجموعة الضابطة.

خلصت هذه الدراسة أن كل من تركيز الهيموغلوبين (Hb) و متوسط حجم الكرية (MCV) و متوسط هيموغلوبين الكرية (MCH) ومتوسط التركيز لهيموغلوبين الكرية (MCHC) و تعداد الخلايا المختلطة (MXD) منخفضة في مرضى السكري مقارنة بالمجموعة الضابطة، بينما أظهرت الدراسة أنه لافرق ذو دلالة إحصائية لكل من تعداد خلايا الدم الحمراء (RBCs) و

الصفائح الدموية (PLTs) ،خلايا الدم البيضاء (WBCs) و حجم الكريات المكدسة (PCV) في مرضى السكري مقارنة بالمجموعة الضابطة.

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Chapter One

Introduction and literature Review

Chapter One

Introduction and literature review

1.1 Introduction

The term diabetes mellitus describes a metabolic disorder of a multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the β-cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (L. Schnack and M.P Romani, 2017).

Complete blood count (CBC) is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or laboratory technician performs the requested testing and provides the requesting medical professional with the results of the CBC. (L. Schnack and M.P Romani, 2017).

Cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions (Buttarello and Plebani, 2008).

1.2Literature review

1.2.1 Characteristics of blood:

Blood defined as vital intravascular fluid circulates throughout heart and blood vessels, and classified as connective tissue. Blood compose of tow portions, solid portion constituted (45) %, consist of white blood cells, red blood cells, and platelets. Fluid portion of the plasma which constituted about 55%. Plasma defined as yellowish fluid in which blood cells suspended and obtained by centrifugation of some portion of anticoagulated blood, plasma contains blood clotting factors (Dean L. 2005). Serum is yellowish fluid obtained from clotted blood and contains some coagulation factors in excepted fibrinogen (Paquette *et al*., 2007). Plasma, which composed of Plasma proteins fats cholesterol triglyceride, lipoproteins vitamins (A,B,C and E) immunoglobulin. Complement proteins (C1-C9). Electrolytes (Na, K, and Cl), trace elements (Fe, Zn, and Mg), enzymes and hormones. Physical properties of blood are including Volume are (5-6) litres in adult. PH (7.34 - 7.45). Saline concentration 9%. Viscosity is 4.5 -5.5 (Hetland *et al*., 2017).

The functions of blood are carrying of gases, nutrition enzymes and. Hormones and immunity agents such as macrophages, microphages, leukocytes, immunoglobulin and complement. Regulation of the P_H, acid base balance and body fluid distribution. Excretions of waste products through execratory organs. Contains coagulation factors, and regulatory mechanism to prevent, loss of blood and thrombosis (Hetland *et al*., 2017).

Evaluation of the blood components perform by Quantitative and qualitative laboratory assessment, to ensure normality or detection of any defect within blood components, is a concept of clinical haematology diagnostic laboratory (Paquette *et al.*, 2007).

1.2.1.1 Hemopoiesis

Defined as a process by which blood cells formed in hemopoietic organs or process by which stem cell developed and differentiated to functional mature blood cells and Induced into the blood stream. During the first few weeks of embryonic life, the formation of blood cells takes place in the yolk sac. Later, until the sixth or seventh month of fetal development, the liver and spleen are the major hematopoietic organs. By the time of birth, more than 90% of all new blood cells are formed in the bone marrow. Here, the progenitor cells are found, in various stages of development, situated in anatomical niches in the bone marrow from where they are then released into the marrow sinuses, the marrow circulation, and further on into the systemic circulation (Paquette *et al.*, 2007).

During infancy and childhood, the marrow of all bones contributes to haematopoiesis. During adult life, hematopoietic marrow is restricted to certain bones (e.g., pelvic bones, vertebral column, proximal ends of the femur, skull, ribs, and sternum. Even in these areas, a proportion of the marrow cavity consists of fat.

During periods of hematopoietic stress (e.g., in severe haemolytic anemias and in some myeloproliferative disorders), the fatty marrow as well as the spleen and liver can resume the production of blood cells. This situation is called extramedullary hematopoiesis (Birbrair *et al* ,.2016).

Growth and differentiation of hematopoietic cells in the bone marrow is regulated by the extracellular matrix and microenvironment provided by stromal cells. These cells, including macrophages, fibroblasts in various stages of differentiation, endothelial cells, fat cells, and reticulum cells, nurture hematopoietic stem cells and progenitor cells by producing growth factors like granulocyte/ macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, or stem cell factor. Other cytokines secreted by

stromal cells regulate the adhesion molecules present on hematopoietic cells, allowing them to remain in the bone marrow or migrate to an area where the respective cell type is needed. All hematopoietic cells of the organism derive from pluripotent stem cells that are capable of both self-renewal and differentiation into all hematopoietic lineages (Morrison and Judith, 2006).

Other cell types such as stromal cells or dendritic cells also derive from the pluripotent hematopoietic stem cell. It has been estimated that one stem cell gives rise to at least 10^6 mature hematopoietic cells. Under normal conditions, the stem cells provide hematopoietic cells for the entire life span. Each day, a healthy adult produces more than 10^{12} hematopoietic cells. Disorders of Stem cells are very rare, representing less than 0.01% of all nucleated cells in the normal bone marrow. Based on animal experiments, the morphology of stem cells is thought to be similar to that of small lymphoid cells. In recent years, the marker expression of human stem cells has been studied. Human stem cells express the surface proteins CD34 and c-kit and are negative for CD38 and lineage-specific markers. In animal systems, stem cells can be assayed as spleen colony-forming units (CFU) in irradiated hosts. Only the more differentiated progenitors of human hematopoietic cells can be tested for their ability to form colonies in soft agar or methylcellulose. One of the earliest progenitor cells in such systems is CFUGEMM, which contains granulocytes, monocytes, erythroid cells, and platelet progenitors (Morrison and Judith, 2006).

From this pluripotent progenitor, more specialized progenitors are formed. Under normal conditions, most stem cells are dormant (G0 phase of the cell cycle). A stem cell divides only to maintain the steady state of haematopoiesis or to meet the body's demand for progenitor cells (stochastic model of haematopoiesis). The daughter cells then either differentiate into determined progenitor cells (e.g., lymph hematopoietic cells) or return to dormancy by re-entering the stem cell pool. Stem cells can be enriched and transplanted (stem cell or bone marrow transplantation).

The stem cell donor does not experience a detectable loss of stem cells (Morrison and Weissman, 1994).

There are several hierarchical levels of stem and progenitor cells. In general, the hematopoietic growth factors do not act on true stem cells, but support the survival and the differentiation of committed cells. Although “early-acting” cytokines such as stem cell factor, FLT3-ligand, G-CSF, or IL-6 regulate the earliest progenitor cells, “late-acting” cytokines such as erythropoietin for erythropoiesis or thrombopoietin for megakaryopoiesis support the growth and differentiation of progenitor cells that are already committed to their respective lineage. Many other cytokines play a positive or negative role in the differentiation of hematopoietic cells (Zhu J and Emerson, 2002).

The gene expression in early stem cells is complex and involves the co-expression of multiple transcription factors. For example, the combination of C/EBP α and Pu 1 directs the expression of the receptor for G-CSF, which is critical for early myelopoiesis. Pu 1 binds to and regulates the promoters of several myeloid growth-factor receptor genes. The Notch family of transmembrane receptors was described in Drosophila as a ligand-dependent suppressor of cell differentiation.

Similar receptors have recently been found on human stem cells, suggesting that they may also be involved in maintaining an undifferentiated state.

The significance of telomeres present in human stem cells and the activity of telomerase in these cells is currently of interest. Telomeres are specialized structures at the end of chromosomes that change with cell division. Shortening of telomeres is associated with cellular aging. Telomerase is an enzyme capable of extending the length of telomeres. It has now been found that adult stem cells have shorter telomeres than fetal stem cells and that the length of telomeres shortens further after transplantation. The activity of telomerase is generally low in stem cells (which corresponds to their quiescent state), but can be up regulated on entry into the cell

cycle. The implications of these findings are not yet clear, but they may indicate that not all stem cells are immortal.

1.2.1.2Erythropoiesis

Red blood cells are specialized cells that deliver oxygen to tissues and remove carbon dioxide from the human body. Erythropoiesis, the "making of red cells," Involves many different genes and gene products that lead to the production of the mature red cell. Erythropoietin begins at the level of the multipotent stem cell, which then undergoes commitment and differentiation. Listed as follows are the stages of erythroid differentiation**:**

1. Stem cell.
2. BFU-E (burst-forming unit, erythroid; immature erythroid progenitor).
3. CFU-E (colony-forming unit, erythroid; more mature erythroid progenitor).
4. Pro-erythroblasts, erythroblasts, normoblasts (morphologically recognizable red Cell precursors, they still have a nucleus, multiply by cell division, and progressively decrease in size as haemoglobin content increases).
5. Reticulocytes; mature red blood cells (erythrocyte).

Remnants of ribosomal RNA can be visualized in reticulocytes; no nucleus is present in the mature red cell. Most nucleated red-cell precursors are confined to the bone marrow.

One pro-erythroblast gives rise to 12–16 mature red blood cells within 5–10 days.

The erythropoietic differentiation is modulated by several cytokines (stem cell factor, IL-3, GM-CSF, and erythropoietin). Erythropoietin is the major cytokine that adapts the production of red cells to the needs of the organism. Both the proliferation and differentiation of CFU-E and late BFU-E are accelerated as a response to erythropoietin. In response to low haemoglobin levels in the blood and tissue hypoxia, the production of erythropoietin by the kidneys is increased (Paquette *et al*., 2007). When the serum levels of erythropoietin are increased, both the rate and

the speed of erythropoiesis increase. Erythropoietin binds to specific receptors on red cell precursors, consequently activating the Janus 2 kinase (JAK2) by tyrosine phosphorylation. This in turn activates the STAT pathway and Ras signal transduction (Palis and Segel, 1998).

A number of transcription factors are involved in the activation of erythroid-specific genes including GATA1, GATA2, NFE2, SCL, EKLF, and myb. During early erythropoiesis, the down regulation of the SCL gene precedes the down regulation of the GATA 2 and GATA 1 genes. In bone marrow, erythropoiesis occurs in distinct anatomic locations called erythroblastic islands, in which a central macrophage is surrounded by a ring of developing erythroblasts (Palis and Segel, 1998).

Important mediators of the cell–cell contact in the erythroid islands include the integrins, the immunoglobulin (Ig) super family, and cadherins. In states of chronic tissue hypoxia (e.g., in haemolytic anemias) the proportion of the marrow devoted to erythropoiesis expands and sometimes transforms a large portion of the fatty marrow into active hematopoietic marrow (Paquette *et al*., 2007).

Requirements for red cells formation are normal healthy erythropoiesis tissues of the bone marrow, proteins, carbohydrate and lipids. Iron and other trace elements. Vitamins (Paquette *et al*., 2007).

1.2.1.3Haemoglobin

Haemoglobin is the molecule responsible for the transport of oxygen. Under physiological conditions, three types of haemoglobins exist:

• Haemoglobin A (α2β2): major adult haemoglobin (96–98%).

• Haemoglobin F (α2γ2): predominant during fetal development, 60–80% at birth, 0.5–0.8% during adult life.

• Haemoglobin A2 (α2δ2): normally 1.5 – 3%.

The haemoglobin molecule has a molecular weight of 64,500 KDa and consists of four polypeptide chains, each carrying a heme group. The heme synthesis starts with the amino acid glycine. Later, porphobilinogen, uroporphyrinogen, coproporphyrinogen and protoporphyrin are formed as intermediate steps. Iron (Fe^{2+}) is supplied from serum transferrin and combines with protoporphyrin to form heme. One heme molecule then binds with one globins chain to form the haemoglobin molecule that avidly binds oxygen (Paquette *et al*., 2007).

The release of oxygen from red cells into tissue is strictly regulated. Under normal conditions, arterial blood enters tissues with an oxygen tension of 90 mmHg and haemoglobin saturation close to 97%. Venous blood returning from tissues is deoxygenated. The oxygen tension in tissues is about 40 mmHg; the haemoglobin saturation is 70–80%. The oxyhemoglobin dissociation curve describes the relation between the oxygen saturation or content of haemoglobin and the oxygen tension at equilibrium. The oxygen dissociation curve has a sigmoid shape. Under normal conditions, only the upper part of this curve is used. The affinity of haemoglobin for oxygen and the deoxygenation in tissues is influenced by temperature, by CO_2 concentration, and by the level of 2,3-diphosphoglycerate in the red cells. In the case of tissue or systemic acidosis, the oxygen dissociation curve is shifted to the right and more oxygen is released. The same effect results from the uptake of carbon dioxide, which raises the oxygen tension of carbon dioxide. This facilitates the unloading of oxygen. As the body temperature increases, the affinity of haemoglobin for oxygen decreases, thereby facilitating oxygen release (Jensen , 2004).

The oxygen supply to peripheral tissues is influenced by three mechanisms:

1. The blood flow, which is controlled by the heart beat volume and the constriction or dilatation of peripheral vessels.
2. The oxygen transport capacity, which depends on the number of red blood cells and the haemoglobin concentration.

3. The oxygen affinity of haemoglobin (Paquette *et al*., 2007).

In anaemic patients, the stroke volume of the heart is increased, the heart beats faster (tachycardia), and, in addition, the 2,3-diphosphoglycerate concentration in red blood cells can increase to facilitate the oxygen dissociation in tissues. A compensation mechanism that takes several days or weeks is the increased synthesis of red blood cells (Paquette *et al*., 2007).

1.2.1.4 The Red Blood Cell

The normal erythrocyte has a diameter of about 8 μm and a biconcave disc form that provides the red cell with a maximum surface-for-gas exchange as well as optimal deformability. The bipolar lipid layer of the red cell membrane is stabilized on the inner side by the attachment of the structural proteins actin and spectrin. Defects of these proteins lead to haemolytic anaemia. The outer layer is covered with mucopolysaccharides that form part of the structure of blood group antigens. The N-acetylneuraminic acid found in these glycoprotein's results in a negative charge of the cell surface (Paquette *et al*., 2007).

Because red cells have lost their nuclei, they are no longer capable of synthesizing proteins, including enzymes. Red cells remain viable and functional for an average of 120 days. The necessary energy for red cell metabolism is supplied by the Embden-Meyerhof pathway, which generates adenosine triphosphate by metabolizing glucose to lactate. This anaerobic process also results in the formation of nicotinamide-adenine dinucleotide, which is essential for the reduction of methemoglobin to functionally active haemoglobin (Paquette *et al*., 2007).

Haemoglobin is split into globin and heme in the reticuloendothelial system. Both components can be recycled. The globin chains are metabolized into amino acids consequently used for the synthesis of new proteins, and iron is used for further heme synthesis. The remaining protoporphyrin is metabolized to bilirubin. The bilirubin is conjugated in the liver and excreted via bile secretions into the intestine.

Intestinal bacteria metabolize bilirubin into stercobilinogen and stercobilin, which are excreted via faeces. Part of these haemoglobin degradation products are reabsorbed and excreted via urine as urobilin and urobiliogen (Paquette *et al*., 2007).

1.2.1.5 Granulopoiesis:

Under the influence of cytokines such as G-CSF, a myeloid progenitor cell, CFU-G, is formed. This cell then differentiates into the morphologically recognizable myeloid precursors: myeloblasts, promyelocytes, myelocytes, and metamyelocytes. Normally these cells do not appear in peripheral blood. Myeloblasts are rather large cells (12–20 μm in diameter) and have a large nucleus with fine chromatin and several nucleoli. No cytoplasmic granules are present. The normal marrow contains up to 5% of myeloblasts. Cell division of myeloblasts results in the formation of promyelocytes, slightly larger neutrophilic precursors with granules in their cytoplasm. These cells in turn give rise by cell division to myelocytes, which have smaller granules (secondary or specific granules). At this stage, a differentiation of the myelocytes into the neutrophil, eosinophil, and basophil series can be recognized (Murphy, 2012). The normal number of neutrophilic granulocytes in the peripheral blood is about 2500–7500/μL. Neutrophilic granulocytes have a dense nucleus split into two to five lobes and a pale cytoplasm. The cytoplasm contains numerous pink blue or gray-blue granules. Two types of granules can be distinguished morphologically: primary or azurophilic granules, which appear at the promyelocyte stage, and secondary granules, which appear later. The primary granules contain myeloperoxidase, acid phosphatase, and acid hydrolases, whereas lysozyme, lactoferrin, and collagenase are found in the secondary granules. All granules are of lysosomal origin (Murphy, 2012).

According to cytokinetic studies, the time required for the division and maturation of a myeloblast to a mature granulocyte is 6–12 days. It has been estimated that 1.5×10^9 granulocytes/kg are produced daily in the healthy organism.

Most of these cells stay at various stages of maturation in the bone marrow, from where they can be mobilized in case of hematopoietic stress. Following their release from the bone marrow, granulocytes circulate for no longer than 12 hours in the blood (Murphy, 2012). Approximately half of the granulocytes present in the bloodstream are found in the circulating pool, whereas the other half is kept in a marinated pool attached to blood vessel walls. After granulocytes move from the circulation into tissues, they survive for about 5 days before they die while fighting infections or as a result of senescence (Murphy, 2012).

The major function of granulocytes (neutrophils) is the uptake and killing of bacterial pathogens. The first step involves the process of chemotaxis, by which the granulocyte is attracted to the pathogen. Chemotaxis is initiated by chemotactic factors released from damaged tissues or complementary components. The next step is phagocytosis or the actual ingestion of the bacteria, fungi, or other particles by the granulocyte. The recognition and uptake of a foreign particle is made easier if the particle is opsonized, which is done by coating them with an antibody or complement. The coated particles then bind to Fc or C3b receptors on the granulocytes. Opsonization is also involved in the phagocytosis of bacteria or other pathogens by monocytes. During phagocytosis a vesicle is formed in the phagocytic cell into which enzymes are released. These enzymes, including collagenases, amino peptidase, and lysozyme, derive from the secondary granules of the granulocyte. The final step in the phagocytic process is the killing and digestion of the pathogen. This is achieved by both oxygen-dependent and –independent pathways. In the oxygen-dependent reactions, superoxide, hydrogen peroxide, and OH radicals are generated from oxygen and NADPH. The reactive oxygen species are toxic not only to the bacteria, but also to surrounding tissue, causing the damage observed during infections and inflammation (Murphy, 2012).

Eosinophils, which make up 1–4% of the peripheral blood leukocytes, are similar to neutrophils but with somewhat more intensely stained reddish granules.

In absolute terms, eosinophils number up to 400/μL. Eosinophilic cells can first be recognized at the myelocyte stage. Eosinophils have a role in allergic reactions, in the response to parasites, and in the defense against certain tumors.

Basophils are seen less frequently than eosinophils; under normal conditions, fewer than 100 cells/μL are found in the peripheral blood. Basophils have receptors for irnmunoglobulin (Ig) E and, in the cytoplasm, characteristic dark granules overlie the nucleus. Degranulation of basophils results from the binding of IgE and allergic or anaphylactic reactions are associated with the release of histamine and heparin (Paquette *et al*., 2007).

Mast Cells Similarly to basophil, mast cells derive from bone marrow CD34+ progenitors, have receptors for IgE, and store histamine. Mast cells typically migrate into and mature in connective tissues. Mast cells participate in allergic and immunological reactions.

As already mentioned, monocytes derive from the myeloid progenitor cell (CFU-GM), which replicates and differentiates into monocytes and, later, macrophages under the influence of certain growth factors. After commitment to the monocytic lineage has been made, the cell goes through distinct monoblast and promonocyte stages before developing into a mature monocyte. Circulating monocytes make up 2–6% of all leukocytes (in absolute numbers 200–800/μL).

Monocytes are larger than most other cells of the blood (diameter 15–20 pm). The cytoplasm is abundant and stains blue, with many fine vacuoles. Fine granules are often present. The nucleus is large and often indented with clumped chromatin. Monocytes and macrophages can phagocytose pathogens, present antigens, and secrete many cytokines (Murphy, 2012).

Macrophages after several hours of transit in the blood, the monocytes migrate into different tissues, where they differentiate into macrophages. Macrophages are larger than monocytes, and have an oval nucleus, prominent nucleoli, a blue cytoplasm, and phagocytic vesicles. The different types of macrophages (e.g., Kupfer cells in the liver, alveolar macrophages in the lung, osteoclasts in the bone, macrophages in the bone marrow, peritoneal macrophages) are known as components of the reticulo-endothelial system. Macrophages are long-lived (life span at least 10 days or much longer) and secrete numerous cytokines, enzymes, and enzyme inhibitors (Murphy, 2012).

The major cytokines secreted by macrophages are tumor necrosis factor (TNF)-α, IL-1α, and IL-1β (monokines). Macrophages avidly phagocytose and kill bacteria and other pathogens. In addition, macrophages have immunological functions (antigen presentation) and can kill tumor cells. An important function of macrophages is to remove debris (scavenger function) and to regulate the proliferation of stromal cells (Murphy, 2012).

1.2.1.6 Lymphatic tissues and immune response

The common or pluripotent hematopoietic stem cell differentiates at an early stage into lymphoid and myeloid progenitor cells. From these lymphoid stem cells, the two main classes of lymphocytes, B- and T-cells, develop. The lymphocytes populate the major lymphatic organs, but can also be found circulating in the peripheral blood. Two types of lymphoid organs can be distinguished: the central lymphoid organs (bone marrow, thymus) and the peripheral lymphoid organs (lymph nodes, tonsils, spleen, and mucosa-associated lymphoid tissue). The central lymphoid organs are the original site of lymphopoiesis and lymphoid maturation, whereas the peripheral lymphoid organs specialize in trapping antigen and initiating adaptive immune responses. In the peripheral blood, 80–85% of the lymphoid cells

belong to the T-cell lineage, whereas in the peripheral lymphoid tissues most lymphoid cells belong to the B-cell lineage.

The major task of B-lymphocytes is the production of antibodies (humoral immunity). The first steps of B-cell differentiation take place in the bone marrow, where lymphoid progenitors differentiate into pro-B- and pre-B-cells. A surface marker that is expressed very early in B-cell ontogeny is CD19. The initial stages of B-cell development depend on the interaction between cell surface molecules and secreted products of stromal cells with their receptor–ligand partners on lymphoid progenitors. Numerous cytokines and growth factors (e.g., TNF, IL-1, IL-2, IL-6, IL-7, IL-10, interferon [IFN]-γ) direct the growth and differentiation of B-cells. In the peripheral blood, where they make up 4–6% of all mononuclear cells, B-cells bear additional surface markers (e.g., CD20, CD22). The specificity of B-cell immunity is reached via surface receptors for antigen (in most cases IgD or IgM). After leaving the bone marrow and circulating in the blood, the mature B-cells migrate into the follicles of the secondary lymphoid organs (e.g., spleen, lymph nodes, and other tissues). Following the contact with an antigen, B-cells differentiate into antibody-secreting plasma cells or into B-memory cells. B-memory cells are long-lived and reside for the most part in lymph nodes. Without antigen stimulation, B-cells have a short life span (Radbruch *et al*., 2006).

The lymphoid follicles provide the necessary environment for B-cells to maintain their existence as mature recirculating antigen-specific cells. The antigen- specific repertoire of B-cells is generated by the sequential rearrangement of Ig gene segments. This developmental program involves changes in the expression of other cellular proteins and is directed by transcription factors. If an intact Ig chain is generated, then this type of rearrangement is terminated, and the next step in the rearrangement cascade can begin. If successive rearrangements fail to generate first a heavy chain (pre-B-cell receptor) and then a light chain, which can be assembled

into a complete immunoglobulin molecule, the B-cell ceases to develop further and goes into apoptosis (programmed cell death). The end result of the successive rearrangements of immunoglobulin genes is a B-cell with a surface Ig of a single specificity (Radbruch *et al*., 2006).

An efficient antibody response and immune defense can only be achieved in cooperation with T-lymphocytes. However, before the T-cells can recognize the antigen and induce the proliferation of B-cells and their differentiation into plasma cells or memory cells, certain criteria must be met. First, antigen-presenting cells must process the antigen (which may be bound to the receptors of B-cells). Next, the processed antigen must be associated with the molecules of the major histocompatibility complex (Radbruch *et al*., 2006).T-lymphocytes also derive from stem cells located in the bone marrow. However, before becoming functional cells, the precursor cells migrate to the thymus where they proliferate, differentiate into mature cells, and are finally released into the blood. At the same time auto reactive T-cells are eliminated. The earliest stage of T-cell development is the prothymocyte. Immature T-cells co-expressing the surface markers CD4 and CD8 are located primarily in the cortex of the thymus. During maturation, the T-cell precursors lose either CD4 or CD8 and migrate to the medulla. A large majority of thymocytes is eliminated during this process. During differentiation, antigen-specific surface receptors are formed (Radbruch *et al*., 2006).

These antigen-receptors recognize either bacterial antigens on antigen-presenting cells or new antigens on tumor cells, transplanted cells, or virally infected cells. Taken together, T-lymphocytes specialize in cell-mediated immunity. They do not react with intact antigens but with antigen fragments presented in association with molecules of the major histocompatibility complex. There are two main classes of T-lymphocytes circulating in the peripheral blood: CD4-positive cells (helper

cells) and CD8-positive cells (suppressor or cytotoxic T-lymphocytes), explained as follows:

• CD4-positive T-lymphocytes recognize foreign antigens in association with HLA class II molecules and have, for the most part, helper or inducer functions.

CD4-positive cells secrete lymphokines after the presentation of the antigen by macrophages has taken place. These cytokines activate macrophages but can also stimulate the proliferation of B-cells and induce the production of antibodies by plasma cells. The secretion of IL-2 also contributes to the development of cytotoxic T-lymphocytes.

• CD8-positive T-lymphocytes react with foreign antigens in association with class I molecules and are the specific effector or killer cells of cell-mediated immunity. CD8-positive lymphocytes also have suppressor functions and control the proliferation of other T-cell subsets as well as the function of B-cells (Radbruch *etal*., 2006).

Natural killer (NK) cells belong to the lymphoid lineage, although some have markers of the monocyte/myeloid lineage. Morphologically, NK cells are characterized as large granular lymphocytes. NK cells are defined by their ability to kill some tumor cells by antigen-independent mechanisms (natural immunity). The physiological function of NK cells is still being debated and includes the removal of certain tumor cells or of virally infected cells. NK cells can be expanded and cultured in the presence of IL-2. Such expanded NK cells (lymphokine-activated killer cells) have been used in the experimental treatment of tumors. Recently, a family of inhibitory receptors on natural killer cells was described. These molecules are specific for some members of the major histocompatibility complex and inhibit the activation of NK cells (Borrego *et al.*, 2002).

1.2.1.7 Megakaryopoiesis

Platelets are small cell fragments (average size 3 – 4 μm) that are important for haemostasis and coagulation. The normal platelet count is between 150,000 and 450,000/μL. Platelets derive from megakaryocytes, which are very large cells with a large, multilobulated nucleus. The mean DNA content of megakaryocytes is at least eight times that of other somatic cells. One megakaryocytic can produce at least several thousand platelets. The formation and release of platelets is related to a preformed structure in the cytoplasm of megakaryocytes, the so-called “demarcation membrane "system." Megakaryocytes derive from megakaryocytic progenitors, which in turn originate in the hematopoietic stem cell. Megakaryocytes are mainly found in the bone marrow but can transit to many organs, including the lung, where part of the platelet release occurs. The maturation of megakaryocytes and the production of platelets occurs under the influence of thrombopoietin. TPO acts, together with certain other cytokines like IL-6 and IL-11, on early megakaryocyte progenitors as well as mature megakaryocytes. Under physiological conditions, the serum levels of TPO are low at normal or elevated platelet counts and high in individuals with low platelet counts (Paquette *et al*., 2007).

1.2.2 Complete blood count:

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC. Alexander Vastem is widely regarded as being the first person to use the complete blood count for clinical purposes. Reference ranges used today stem from his clinical trials in the early 1960s.(www.healthdirect.gov.au/full-blood-count).

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdiction (Maton, Anthea *et al* ., 1993).A phlebotomist collects the specimen, in this case blood is drawn in a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting, and transported to a laboratory. In the past, counting the cells in a patient's blood was performed manually, by viewing a slide prepared with a sample of the patient's blood under a microscope (a blood film, or peripheral smear). Nowadays, this process is generally automated by use of an automated analyzer, with only approximately 30% samples now being examined manually. (www.healthdirect.gov.au/full-blood-count).

1.2.2.1Automated blood count

The blood is well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review. Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Within this tubing, there are sensors that count the number of cells going through it, and can identify the type of cell; this is flow cytometry. The two main sensors used are light detectors, and electrical impedance. One way the instrument can tell what type of blood cell is present is by size. Other instruments measure different characteristics of the cells to categorize them. (www.healthdirect.gov.au/full-blood-count).

Because an automated cell counter samples and counts so many cells, the results are very precise. However, certain abnormal cells in the blood may be identified incorrectly, and require manual review of the instrument's results and identifying any abnormal cells the instrument could not categorize (Maton, Anthea *et al*.,1993).

In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated hematology analyzers also measure the amount of hemoglobin in the blood and within each red blood cell. This information can be very helpful to a physician who, for example, is trying to identify the cause of a patient's anemia. If the red cells are smaller or larger than normal, or if there's a lot of variation in the size of the red cells, this data can help guide the direction of further testing and expedite the diagnostic process so patients can get the treatment they need quickly (Maton, Anthea *et al.*, 1993).

1.2.2.2 Manual blood count

Counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) are used to calculate the number of red and white cells per litre of blood. To identify the numbers of different white cells, a blood film is made, and many white cells (at least 100) are counted. This gives the percentage of cells that are of each type. By multiplying the percentage with the total number of white blood cells, the absolute number of each type of white cell can be obtained. (www.healthdirect.gov.au/full-blood-count).

The advantage of manual counting is that automated analysers are not reliable at counting abnormal cells. That is, cells that are not present in normal patients and are only seen in the peripheral blood with certain haematological conditions. Manual

counting is subject to sampling error because so few cells are counted compared with automated analysis (Maton and Anthea *et al.*,1993).

Medical technicians examine blood film via a microscope for 30% of CBCs, not only to find abnormal white cells, but also because variation in the shape of red cells is an important diagnostic tool. Although automated analysers give fast, reliable results regarding how many red cells, the average size of the red cell, and the variation in size of the red cells, they don't detect cells' shapes. Also, some normal patients' platelets will clump in EDTA anticoagulated blood, which causes automatic analyzers to give a falsely low platelet count. The technician viewing the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets (Maton and Anthea*et al.*,1993).

•A complete blood count will normally include:

•Red cells

- Total red blood cells — The number of red cells is given as an absolute number per litre.
- Haemoglobin - The amount of hemoglobin in the blood, expressed in grams per decilitre. (Low hemoglobin is called anaemia.)
- Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells.
- Red blood cell indices
    - Mean corpuscular volume (MCV) - the average volume of the red cells, measured in femtolitres. Anaemia is classified as microcytic or macrocytic based on whether this value is above or below the expected

normal range. Other conditions that can affect MCV include thalassemia, reticulocytosis and alcoholism.

- Mean corpuscular haemoglobin (MCH) - the average amount of haemoglobin per red blood cell, in picograms.
- Mean corpuscular haemoglobin concentration (MCHC) - the average concentration of haemoglobin in the cells.

Red blood cell distribution width (RDW) - a measure of the variation of the RBC population. (www.healthdirect.gov.au/full-blood-count).

•White cells

- Total white blood cells - All the white cell types are given as a percentage and as an absolute number per liter.

Differential leucocytes counts will also include:

- Neutrophil granulocytes — May indicate bacterial infection. May also be raised in acute viral infections. Because of the segmented appearance of the nucleus, neutrophils are sometimes referred to as "segs." The nucleus of less mature neutrophils is not segmented, but has a band or rod-like shape. Less mature neutrophils — those that have recently been released from the bone marrow into the bloodstream — are known as "bands" or "stabs". Stab is a German term for rod.
- Lymphocytes — Higher counts with some viral infections such as infectious mononuclesis and. Also raised in chronic lymphocytic leukaemia (CLL). Can be decreased by HIV infection. In adults, lymphocytes are the second most common WBC type after neutrophils. In young children under age 8, lymphocytes are more common than neutrophils..

- Monocytes — May be raised in bacterial infection, tuberculosis, malaria, Rocky Mountain spotted fever, monocytic leukaemia, chronic ulcerative colitis and regional enteritis .
- Eosinophil granulocytes — increased in parasitic infections, asthma, or allergic reaction.
- Basophil granulocytes- May be increased in bone marrow related conditions such as leukaemia or lymphoma.

A manual count will also give information about other cells that are not normally present in peripheral blood, but may be released in certain disease processes (Maton, Anthea *et al.*,1993).

•Platelets

- Platelet numbers are given, as well as information about their size and the range of sizes in the blood.
- Mean platelet volume (MPV) - a measurement of the average size of platelets.

Many disease states are heralded by changes in the blood count:

- leukocytosis can be a sign of infection.
- thrombocytopenia can result from drug toxicity.
- pancytopenia is generally as the result of decreased production from the bone marrow, and is a common complication of cancer chemotherapy (Maton and Anthea , *et al.*,1993).

1.2.3 Diabetes mellitus:-

Definition The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances

of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and eatures of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. (L Schnack and M.P Romani, 2017).

1.2.3.1 Diagnosis and diagnostic criteria:

If a diagnosis of diabetes is made, the clinician must feel confident that the diagnosis is fully established since the consequences for the individual are considerable and lifelong. The requirements for diagnostic confirmation for a person presenting with severe symptoms and gross hyperglycaemia differ from those for the asymptomatic person with blood glucose values found to

be just above the diagnostic cut–off value. Severe hyperglycaemia detected under conditions of acute infective, traumatic, circulatory or other stress may be transitory and should not in itself be regarded as diagnostic of diabetes. The diagnosis of diabetes in an asymptomatic subject should never be made on the basis of a single abnormal blood glucose value. For the asymptomatic person, at least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either fasting, from a random (casual) sample, or from the oral glucose tolerance test (OGTT). If such samples fail to confirm the diagnosis of diabetes mellitus, it will usually be advisable to maintain surveillance with periodic re–testing until the diagnostic situation becomes clear. In these circumstances, the clinician should take into consideration such additional factors as ethnicity, family history, age, adiposity, and concomitant disorders, before deciding on a diagnostic or therapeutic course of action. An alternative to blood glucose estimation or the OGTT has long been sought to simplify the diagnosis of diabetes. Glycated haemoglobin, reflecting average glycaemia over a period of weeks, was thought to provide such a test. Although in certain cases it gives equal or almost equal sensitivity and specificity to glucose measurement, it is not available in many parts of the world and is not well enough standardized for its use to be recommended at this time. (Alberti and Zimmet, 1998). The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases, drowsiness and coma; high levels of glycosuria are usually present. (WHO, 1985).

For clinical purposes, an OGTT to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range (i.e. between the levels that establish or exclude diabetes) and fasting blood glucose levels are below those which establish the diagnosis of diabetes. If an

OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75-g oral glucose load. For children, the oral glucose load is related to body weight: 1.75g per kg. The diagnostic criteria in children are the same as for adults.

1.2.3.2 Classification:

The classification encompasses both clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia, as suggested by Kuzuya and Matsuda. The clinical staging reflects that diabetes, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology. The classification by aetiological type results from improved understanding of the causes of diabetes mellitus (Kuzuya and Matsuda, 1997).

1.3.Previous studies:

A previous study showed that the decreases of hemoglobin concentration don't reach to the significant level. The difference was statistically significant in mean of WBC count in diabetic patients compare to control (Al-Ali, 2016). Another study in Mecdonia which investigated the differences in hematological and biochemical parameters in type I diabetic retinopathy patients and type II diabetic patients showed an increased in hematocrit, leucocyte, lymphocyte and monocyte number in diabetic patients, while MCV, MCH(affected of the glucose concentration) and neutrophils number are decrease. Patients with type 1 diabetes showed an increase in erythrocytes, HCT, leucocytes, lymphocytes and glucose concentration compared with the control. Hematocrit and platelets index

(PDW, MPV, PLCP) are significantly increased while neutrophils and monocytes are decreased in patients with type 1 diabetes and AMI in relation with type 2 diabetes. These suggests that platelet hyperfunction in diabetic patients may by implicated in the athogenesis of diabetic retinopathy. These results established an altered in platelets volume indices in insulin dependent diabetics suggesting that platelets may involve in developing micro and macro vascular complication in patients (Gruev *et al*.,2011).

A recent study in Bangladesh, about diabetes type II and RBCs parameters concluded that RBC count, PCV, MCV as an RBC index are down rising and MCHC, RDW are up rising as a new marker associated with higher mortality in health and disease. In diabetic patients, the glycemic control does affect the MCHC and RDW. Good glycemic controls associated with lower RDW and MHC value than in patients with poor control. Both MCHC & RDW are directly and significantly associated. This positive correlation may strengthen the notion that RDW is an inflammatory marker. The study in our population can be considered as an initial one that necessitates further studies to define the relation between RBC count, PCV, MCV, MCHC and RDW with different diabetic complications and its predictive value. Further studies are also required to define specific values of the RDW to indicate specific risks in diabetic patients. (Jaman *et al.,* 2018)

Justification

The incidence of diabetes mellitus in Sudan is increasing and contributes significantly to total hospital morbidity and mortality. Mohamed and Hassan, (2001). According to the latest WHO data published in April 2011 Diabetes Mellitus Deaths in Sudan reached 8,003 or 2.17% of total deaths. The age adjusted Death Rate is 38.76 per 100,000 of population in Sudan. (WHO. 2011)

Anaemia is a key indicator of chronic kidney disease (CKD) but occurs earlier during diabetic kidney disease and may be more severe than previously realized in patients with diabetes (Vlagopoulos *et al*., 2005).

Most patients with diabetes are rarely tested for anaemia and are unaware of the link between anaemia and kidney disease. (Stevens *et al*., 2003).

Elevated WBC count, even within the normal range, is associated with both macro- and microvascular complications in type 2 diabetes. Chronic inflammation, as indicated by a higher WBC count, may play a linkage role in the development of macro- and microvascular complications in diabetes. (Tong *et al*., 2004).

In a recent study in Sudan was done to investigate the association between HbA1c and Hb/RBCs count and RBCs indices; specifically, Hb/RBCs count and indices in non- diabetic pregnant women, In general, features of iron deficiency anemia involve a reduction in RBCs indices *e.g.* MCV, MCH, and MCHC.39 Thus, as the Hb content inside a single RBC is reduced due insufficient amounts of iron, subsequently the concentration of the Hb in the total mass of the RBCs will be reduced too and this will affect HbA1c value. (Abass *et al*., 2017)

Objectives

General objective:

-To determine the Effects of Diabetes Milletus Type II on the Complete Blood Count among Sudanese patients.

Specific objectives:

- To study the effects of Diabetes Milletus Type II on full blood count pictures.
- To study the effects of Diabetes Milletus Type II on WBCs and platelets count.
- To study the effects of medication on Diabetes Milletus Type II on Hb levels.

Chapter Two

Materials and Methods

Chapter two

Material and methods

3.1. Study design and area:

This was a cross sectional study design based on whole blood sample of 100 diabetic patients and 50 non diabetic people in ALshaheeda Nada health centre in Al Haj Yousif neighborhood in the period between February 2016 and March 2017, to know the effects of diabetes milletus on complete blood count.

3.2. Study population:

A total of 150 whole blood samples were collected, 100 subjects as cases and 50 subjects as controls.

3.3. Inclusion Criteria

Case group:

A Known diabetic patient attending ALshaheda Nada health centre (Mar-APR 2017), subjects were asked verbally about previous diagnosis of diabetes mellitus.

Control group:

A non diabetic healthy person attending ALshaheda Nada health centre (Mar-APR 2017)

3.4. Exclusion Criteria:

Any participant did not imply the inclusion criteria above.

3.5. Blood Sampling:

A 2.5 ml of EDTA vinous blood was taken. The sample was then sent as early as possible (maximum 3 to 6 hours) to Modern Medical Analysis Centre for analysis. For hematological parameters a standard coulter gram was done on the sysmex Counter.

3.6. Data collection tools:-

The primary was collected by using questionnaire.

3.7. Methods:

Complete blood count (CBC):

3.7.1. Principle of sysmex:

The SysmxXE_2100 is haematology automated analyzer used to quickly perform full blood counts and it made by Sysmex Corporation Principles of measurement (Japan).

Diluted blood is pass through a tube which thin enough that can pass cells by one at a time, characteristic about the cell are measured using lasers or electrical impedance.

3.7.2. Procedure:

Vinous blood was collected in EDTA container and analyzed for different haematological parameters (Hb, PCV, RBCs, WBCs, platelet count, RBCs indices). The data entry and analysis was done on computer package SPSS (Statistical Packages of Social Sciences) version 16.0. The results were given in the text as mean, standard deviation and 95% confidence intervals of haematological values (complete blood count).

3.8. Result interpretation:

All quality control measures were adopted during specimen collection and processing.

3.9. Data analysis:

The data were compared by using statistical analysis performed with Statistical Package for Social sciences (SPSS) software version 16. To compare means and standard deviation of haematological values, between normal people and known diabetic patients, and parity Student's t-test was used. In all statistical analysis, only $p<0.05$ were considered significant.

3.10. Ethical considerations:

Procedure of whole blood sampling was explained to the participants. All participants were informed verbally about the research objectives and procedures during the interview period.

Chapter Three

Results

Chapter three

Results

3.1 General characteristics of the study population:

This study was conducted on 150 subjects, from them, 56 (37%) were males and 94 (63%) were females (Figure 4.1). The age of the subjects ranged between 40-85 years old with mean age of 59 ± 11.6 years old.

The age groups were dividedinto 40-49, 50-59, 60-69 and more than 70 years old. The age groups with highest frequency of was 60-90(34%), followed by age group 50-59 (31%), the age groups 40-49 and more than 70 years have lower percentages 19 % and14 % respectively (Figure4.2)**.**

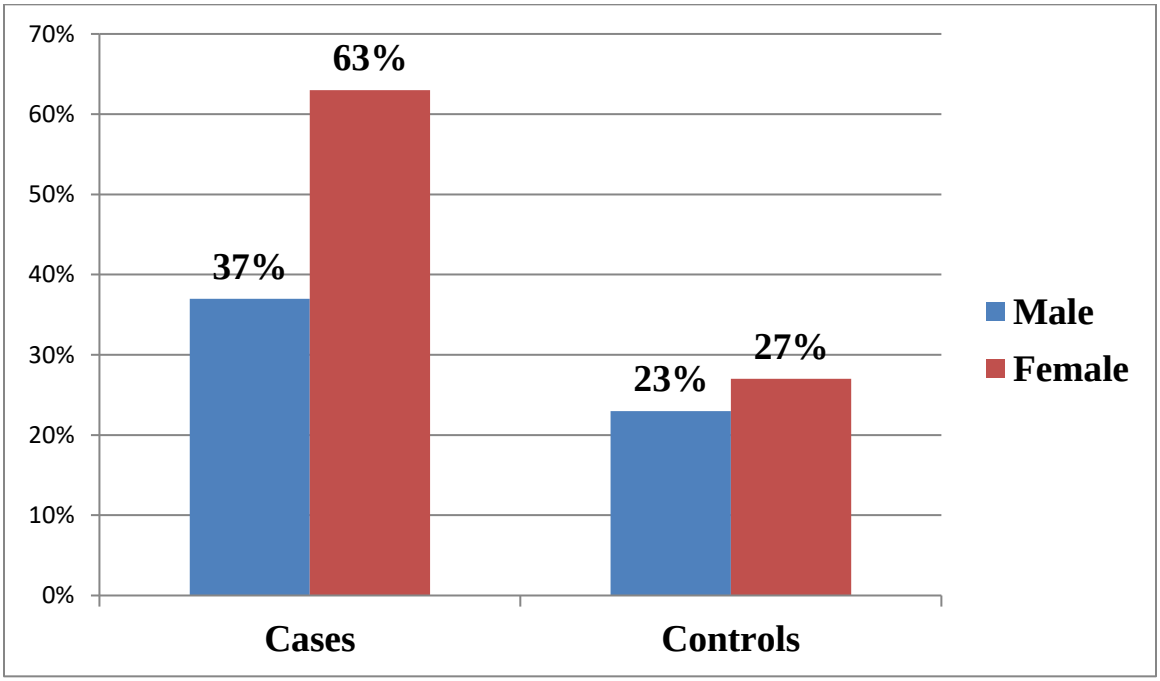

Figure {4-1} Frequency of gender among diabetic cases, Females (63%) and males (37%).

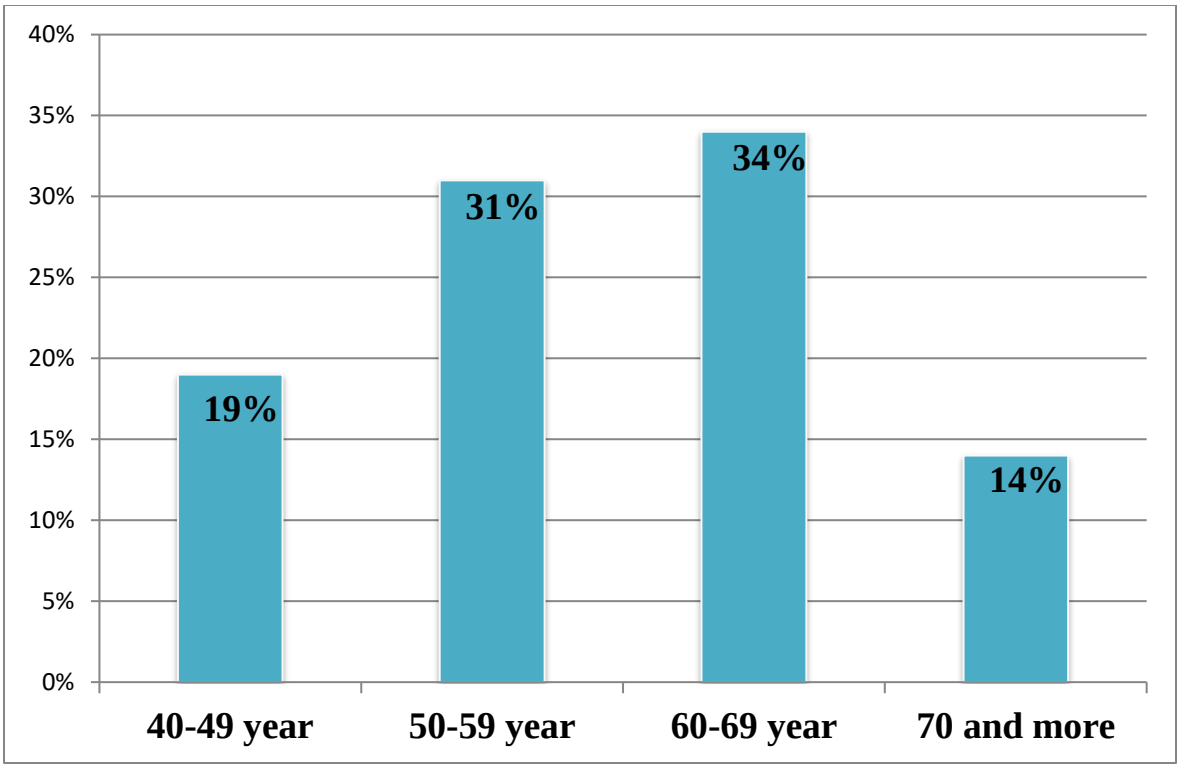

Figure {4-2} Frequency of age groups among diabetic cases.

From figure (1) we note that the age of most of the individuals study are (60-69 year) by (34)and with (34%) while the total number is (50-59 year) by (31) and with (31%).

3.2 The relation between CBC parameters among cases and controls:

-There was statistically significant decrease in hemoglobin concentration, MCV, MCH and MCHC (*P.value* =0.00, 0.02, 0.01, 0.00 respectively) and no statistically significant change in RBCs and HCT (*P.value* = 0.34 and 0.34 respectively) in DM type II patients when compared with healthy controls. Table (4-1).

Table (4-1): The mean differences of the TRBCs, heamoglobin and indices among cases and controls.

Variable	Subjects	Mean ± SD	p-value
Hemoglobin	Control	13.59 ± 0.47	0.00
	Case	12.77 ± 1.42	
RBCs	Control	4.58 ± 0.36	0.34
	Case	4.68 ± 0.63	
HCT	Control	42.61 ± 2.92	0.34
	Case	42.04 ± 3.71	
MCV	Control	93.08 ± 3.357	0.02
	Case	91.02 ± 5.98	
MCH	Control	28.54 ± 1.32	0.01
	Case	27.61 ± 2.31	
MCHC	Control	32.52 ± 2.08	0.00
	Case	30.34 ± 1.79	

-There was statistically significant increased in MXD count (*P. value* =0.04) with no statistically significant difference in platelets, WBCs, lymphocytes and neutrophil counts (*P. value* = 0.57, 0.09, 0.99, 0.51 respectively) when compared with healthy control. Table (4-2).

Table (4-2): The mean differences of the platelets, TWBCs and differential counts among cases and controls.

Variable	Subjects	Mean ± SD	p-value
PLT	Control	2.56 ± 52.34	0.57
	Case	2.61 ± 57.49	
WBC	Control	5.75 ± 0.97	0.09
	Case	6.22 ± 1.79	
LYM	Control	37.98 ± 6.43	0.99
	Case	37.97 ± 10.29	
MXD	Control	7.78 ± 2.41	0.04
	Case	9.01 ± 3.94	
NEUT	Control	54.21 ± 7.05	0.51
	Case	53.02 ± 11.73	

Chapter Four

Discussion, Conclusion and Recommendations

Chapter four

Discussion

The previous study showed that patients with T2DM had lower hemoglobin concentrations than the control group, but still within the normal range of hemoglobin level. This study showed a strong association between Hb and T2DM (*p-value*=0.000), this result was comparable to study done in Iraq, which revealed Hb = 9.7 g/dl (Al-Ali, 2016).

This study showed insignificant association in the mean of RBCs count. A previous study done in Saudi Arabia showed strong significant decrease in mean of T2DM (Al-Shehri, 2017).

In the present study, HCT levels showed insignificant association (*p-value*=0.343), which disagreed with Al-Ali's study (p-value <0.05) (Al-Ali, 2016).

This study showed significant associations in MCV, MCH and MCHC (*p-value* <0.05), this was like other study done in Saudi Arabia, which revealed significantly decreased values in RBCs counts and indices in patients with T2DM. (Al-Shehri, 2017)(Al-Shehri, 2017).

In the present study, the means of TWBCs, lymphocytes and neutrophils were statistically highly insignificant (p-value > 0.05), while the mean mixed cells count was statistically significant(p-value<0.05), these results were different than a previous study done Sudan among patients with the type two diabetes, which showed no statistically significant values (p-value > 0.05) (Tahir ,2015). While the platelets count in this study was statistically insignificant (p-value > 0.05), this was disagreed with a previous study done in Nigeria which showed significant values (*p-value* < 0.05) (Akinsegun *et al*., 2014).

Conclusion

- CBC parameters HB, MCV, MCH, MCHC and Mixed count are significantly lower among diabetic subjects compared to apparently healthy controls. This reflects poor glycemic control and lifestyle changes.
- No statistically significant difference on the other parameters namely RBC, WBC, PLT and PCV.

Recommendations

- Further studies should be done with a larger sample size.
- Further studies should be done to measure other parameters such as FBG, HbA1C and RFT in addition to CBC parameters to reach a better assessment of DM effects on the hematological profile of the patients.

References

References

1. **Abass, A. E., Musa, I. R., Rayis, D. A., Adam, I., & Gasim I, G. (2017).** Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. Clinics and practice, 7(4), 999.

2. **Akinsegun, A., Akinola Olusola, D., Sarah, J-O., Olajumoke, O., Adewumi, A., Majeed, O., Anthonia, Ogbera, E., Uche, Olaitan, Okunoye, O., Arogundade and Kingsley, A. (2014).** Mean platelet volume and platelet counts in type 2 Diabetes: Mellitus on treatment and non-diabetic mellitus controls in Lagos, Nigeria. The *Pan African Medical Journal*, 18,42.

3. **Al-Ali, Z. A. J . (2016).** Some hematological and biochemical parameters in type 2 diabetic patients Missan/ Iraq. *Int. J. Adv. Res. Biol. Sci*. **3**(4): 30-34.

4. **Alberti K.G.M.M. and Zimmet P.Z. (1998)**. Definition, Diagnosis and Classicization of Diabetes Mellitus and its Complication Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation, *Diabetic Medicine*,**15**:539-553.

5. **Birbrair, Alexander; Frenette, Paul S. (2016).** "Niche heterogeneity in the bone marrow". *Annals of the New York Academy of Sciences*. **1370**: 82-96. doi:10.1111/nyas.13016.

6. **Buttarello, M; Plebani, M (2008).** "Automated blood cell counts: state of the art". *American Journal of Clinical Pathology*. **130**(1): 104–16.

7. **Dean L. (2005).** Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); Chapter 1, Blood

and the cells it contains. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2263/

8. **Gruev, T., Janike vikIvanovska, D., Jovcevska-Mecevska, J., & Dimitrov, S. (2011).** Differences in hematology and biochemical parameters in diabetic patients.*Clin Chem Lab Med*; 49, Special Suppl, pp S1 – S874.

9. **Hetland, Ctaliv Eline; Susrud, Kjærsti Sørensen; Lindahl, Kim Hein; Bygum, Anette. (2017).** Henoch-Schönlein Purpura: A Literature Review. *Acta Dermato-Venereologica*, **97**:8-10, pp. 1160-1166(7).

10. **Jaman M.S., Rahman M.S., Swarna R.R., Mahato J., Miah M.M., and Mosa. Ayshasiddeka (2018).** Diabetes and red blood cell parameters. *Ann Clin Endocrinol Metabol*.; **2**: 001-009.

11. **Jensen F.B. (2004).** Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O2 and CO2 transport. Acta *Physiologica Scandinavica*;**182**(3):215-27.

12. **Kuzuya T and Matsuda A. (1997).** Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. *Diabetes Care;***20**:219-2-.

13. **L. Schnack L., MP Romani A. (2017).** The Metabolic Syndrome and the Relevance of Nutrients for its Onset. Recent patents on biotechnology. 1;**11**(2):101-19.

14. **Maton, Anthea; Jean, H., Charles, W., Susan, J., Maryanna, W., David, L., Jill, D., Wright. (1993).** Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall ISBN 0-13- 981176-1**.**

15. **Mohamed Ahmed A., Hassan Ahmed N. (2001).** Diabetes mellitus in Sudan: the size of the problem and the possibilities of efficient care. *Practical Diabetes International*; **18**(9):324-7.

16. **Morrison, J.; Judith Kimble (2006).** "Asymmetric and symmetric stem-cell divisions in development and cancer". Nature. **441** (7097): 1068–74.

17. **Morrison, SJ; Weissman, IL (1994).** "The long-term repopulating subset of hematopoietic stem cells is deterministic and isolable by phenotype.". Immunity. **1** (8): 661–73.

18. **Murphy, P., (2012).** The neutrophil. Springer Science & Business Media.

19. **Palis J, Segel GB (1998).** "Developmental biology of erythropoiesis". Blood Rev. **12** (2): 106–14.

20. **Paquette R., Munker R. (2007).** Transplantation of Stem Cells From Bone Marrow, Peripheral Blood, and the Umbilical Cord. In: Munker R., Hiller E., Glass J., Paquette R. (eds) Modern Hematology. Contemporary Hematology. Humana Press.

21. **Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dörner T, Hiepe F. (2006).** Competence and competition: the challenge of becoming a long-lived plasma cell. *Nature Reviews Immunology* ;**6**(10):741.

22. **Stevens, P.E., O'Donoghue, D.J. and Lameire, N.R., (2003).** Anaemia in patients with diabetes: unrecognised, undetected and untreated?. Current medical research and opinion, **19**(5), pp.395-401.

23. **Tahir, Safa. Abd Al Majed Said and Nadia M. (2015).** Total count and absolute values of White Blood Cells in patients with Diabetes Mellitus Type 2. Sudan University of Science and Technology- College of Graduate Studies**.**

24. **Tong, P.C., Lee, K.F., So, W.Y., Ng, M.H., Chan, W.B., Lo, M.K., Chan, N.N. and Chan, J.C., (2004).** White blood cell count is associated with macro- and microvascular complications in Chinese patients with type 2 diabetes. *Diabetes care*, 27(1), pp.216-222.

25. **Vlagopoulos, P.T., Tighiouart, H., Weiner, D.E., Griffith, J., Pettitt, D., Salem, D.N., Levey, A.S. and Sarnak, M.J., (2005).** Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease. *Journal of the American Society of Nephrology*, 16(11), pp.3403-3410.

26. **World Health Orgnaization (1985).** Diabetes Mellitus: Report of a WHO Study Group. Geneva: WHO. Technical Report Series 727.

27. **World Health Orgnaization (2011).** Diabetes Mellitus: Report of Deaths in Sudan.

28. www.healthdirect.gov.au/full-blood-count

29. **Zhu J, Emerson SG (2002).** Hematopoietic cytokines, transcription factors and lineage commitment. Oncogene; **21**(21):3295.

Appendix

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The Effects of Diabetes Milletus Type-II on the Complete Blood Count among Sudanese patients- Khartoum State

Questionnaire

Serial No (….)

Demographical data:

-Age: …… years.

-Gender: Female (…..) Male (….)

-Diagnosis……

-Medication…….

Laboratory Results:

Complete Blood Count parameters:

-TWBCs……$\times 10^3/\mu l$.

-RBCs……$(10^6/\mu l)$.

-Platelets count……..$(\times 10^3/\mu l)$.

-Hb …….(mg/dl)

-HCT ……(mg/dl)

-MCV……..(fl).

-MCH……. (Pg)

-MCHC…….(mg/dl)

-Lymphocytes ……..$(\times 10^3/\mu l)$

-MXD……$(\times 10^3/\mu l)$

-Neutrophils……$(\times 10^3/\mu l)$

List of abbreviations

Abbreviations	Term
CBC	Complete Blood Count
DM	Diabetes Mellitus
Hb	Hemoglobin
RBCs	Red Blood Cells
PCV	Packed Cell Volume
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
WBCs	White Blood Cells
MXD	Mixed cells
LYM	Lymphocytes
NET	Neutrophils
PLTs	Platelets
EDTA	Ethylediaminetetraacetic acid
GM-CSF	Granulocyte/ Macrophage Colony-Stimulating Factor
G-CSF	Granulocyte Colony-Stimulating Factor
CFU	Colony-Forming Units
BFU-E	Burst-Forming Unit, Erythroid
NK cells	Natural killer cells
RDW	Red Blood Cell Distribution Width
CLL	Chronic Lymphocytic Leukaemia
OGTT	Oral Glucose Tolerance Test