Chapter One

Introduction and Literature Review

1.1 Introduction

Pregnancy is the condition of having a developing embryo or fetus in the female body, after union of an oocyte (ovum) and spermatozoon. The average gestation period for a human pregnancy is 38 weeks from the first day of the last menstrual period.

Conception: Once a month an ovum (secondary oocyte) matures in one of the ovaries and travels down the nearby fallopian tube to the uterus; this process is called ovulation. At fertilization, which must take place within a day or two of ovulation, one of the spermatozoa unites with the ovum to form a zygote. The zygote then implants itself in the wall of the uterus, which is richly supplied with blood, and begins to grow. It is conventionally divided into three trimesters, each roughly three months long.

A condition of pregnancy characterized by a reduction in the concentration of hemoglobin in the blood. It may be physiologic or pathologic. In physiologic anemia of pregnancy, the reductions in concentration result from dilution because the plasma volume expands more than the erythrocyte volume. The hematocrit in pregnancy normally drops several points below its pregnancy level. In pathologic anemia of pregnancy, the oxygen-carrying capacity of the blood is deficient because of disordered erythrocyte production or excessive loss of erythrocytes through destruction or bleeding. Pathologic anemia is a common complication of pregnancy, occurring in approximately half of all pregnancies. Disordered production of erythrocytes may result from

nutritional deficiency of iron, folic acid, or vitamin B_{12} or from sickle cell or another chronic disease, malignancy, chronic malnutrition, or exposure to toxins. Destruction of erythrocytes may result from inflammation, chronic infection, sepsis, autoimmune disease, microangiopathy, or a hematologic disease in which the erythrocytes are abnormal. Excessive loss of erythrocytes through bleeding may result from abortion, bleeding hemorrhoids, intestinal parasites such as hookworm, placental abnormalities such as placenta previa and abruption placentae, or postpartum uterine atony (Williams and Wilkins, 2006).

In previous study, assessment of complete blood count of pregnant women in Port Sudan city by Khalil and Rania, publishing by Sudan University of Science and Technology. The results indicated that WBC of pregnant women with number of pregnancy between (1-3) pregnancies increased insignificantly compared to those of pregnancy between (4-7) and (7-10).WBC of pregnant women at third trimester increased insignificantly while lymphocytes decreased significantly than those women at first and second trimesters. Neutrophils of women at third trimester increased significantly compared to those in first and second trimesters, when compared of different trimesters with control the result was WBCs and neutrophils increased significantly at third trimester but for lymphocytes was decreased significantly at third trimester. MPV of pregnant women with history of abortion significantly increased compared to those with no history of abortion. RBC and HCT of pregnant women increased significantly with regularly visits to clinics while basophils decreased insignificantly compared to those with irregular visits clinics.

1.2 Literature Review

1.2.1. Hematopoiesis

Hematopoiesis is defined as the production, development, differentiation, and maturation of all blood cells. Within these four functions is cellular machinery that outstrips most high-scale manufacturers in terms of production quotas, customs specifications, and quality of final product (Ciesla, 2007).

1.2.1.1. Site of haemopoiesis

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis, and from 6 weeks until 6-7 months of fetal life the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth. The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation. In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri. Even in these haemopoietic areas, approximately 50% of the marrow consists of fat. The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extramedullary haemopoiesis').In fetus 0-2months yolk sac, 2-7 months liver and spleen, 5-9 months bone marrow. In infants bone marrow practically all bones. In adults vertebrae,

ribs, sternum, skull, sacrum and pelvis, proximal ends of femur (Hoffbrand *et al*., 2006).

1.2.1.2. **Haemopoietic stem and progenitor cells**

Haemopoiesis starts with a pluripotential stem cell that can give rise to the separate cell lineages. This haemopoietic stem cell on Immunological testing it is $CD34^+$ $CD38^-$ and has the appearance of a small or medium-sized lymphocyte. Cell differentiation occurs from the stem cell via the committed haemopoietic progenitors which are restricted in their developmental potential. An example is the earliest detectable mixed myeloid precursor which gives rise to granulocytes, erythrocytes, monocytes and megakaryocytes and is termed CFU (colony-forming unit)-GEMM .The bone marrow is also the primary site of origin of lymphocytes which differentiate from a common lymphoid precursor. The stem cell has the capability for self-renewal so that marrow cellularity remains constant in a normal healthy steady state. There is considerable amplification in the system: one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions. The precursor cells are, however, capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises (Hoffbrand *et al.,* 2006).

1.2.1.3. Bone marrow stroma

The bone marrow forms a suitable environment for stem cell survival, growth and development. It is composed of stromal cells and a micro vascular network. The stromal cells include adipocytes, fibroblasts, endothelial cells and macrophages and they secrete extracellular molecules such as collagen, glycoproteins and glycosaminoglycans to form an extracellular matrix. In addition, stromal cells secrete several growth factors necessary for stem cell survival. Mesenchymal stem cells

are thought to be critical in stromal cell formation. Stem cells are able to traffic around the body and are found in peripheral blood in low numbers. In order to exit the bone marrow, cells must cross the blood vessel endothelium and this process of mobilization is enhanced by administration of cytokines such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF).The reverse process of stem cell homing appears to depend on a chemokine gradient in which the stromal derived factor (SDF-l) is critical. Several critical interactions maintain stem cell viability and production in the stroma including stem cell factor (SCF) and Jagged proteins expressed on stroma and their respective receptors c-Kit and Notch expressed on stem cell (Hoffbrand *et al*., 2006).

1.2.1.4. Erythropoiesis

Red cells are produced by proliferation and differentiation of precursors whose dominant representatives in the bone marrow are the erythroblasts. Erythroblasts are referred to as normoblasts when their morphological features are within normal limits. During the course of differentiation, the size of erythroblasts progressively decreases, and the character of the nucleus and cytoplasm changes as the cells proceed toward the point where proliferative capacity is lost and hemoglobin becomes the predominant protein in the cytoplasm (Firkin *et al.*, 1989).

1.2.1.5. Granulopoiesis

The predominant white blood cell, or leukocyte, in the circulation is the mature granulocyte. The color of the numerous granules in the cytoplasm after staining with Romanov sky stains is the basis of the classification of granulocytes into neutrophil, eosinophil and basophil series. This distinction is important, as the mature forms of the different. Granulocyte series perform different roles. Neutrophils are by far the most common

circulating form. of granulocyte, and play an essential role in phagocytosing and killing invading microorganisms. Eosinophils and basophils perform separate functions in inflammatory processes. Mature granulocytes are produced by the proliferation and maturation of precursors from the earliest recognizable stage, the myeloblast, through the promyelocyte, myelocyte, metamyelocyte and stab - form stage, until the mature segmented stage is reached (Firkin *et al*., 1989).

1.2.1.6. Formation of monocytes and macrophages

The monocyte – macrophage and granulocytic series collectively constitute the myeloid series, whose mature forms are the most important mobile phagocytic cells involved in host defense against infection. Mature monocytes have less vigorous phagocytic capacity and a longer lifespan than segmented neutrophils. They are able to re-enter the circulation, but are primarily distributed in the extra vascular space. The macrophages, and the multi-nucleated giant cells to which they give rise, are distributed in the extra vascular space. Macrophages located in lymph nodes, liver, spleen and. bone marrow is an integral part of the reticuloendothelial system, which ingests and degrades both foreign and damaged autologous material (Firkin *et al*., 1989).

1.2.1.7. Lymphopoiesis

Animal studies indicate that the lymphocytes which are present in foci in the bone marrow and in the thymus are engaged in particularly rapid proliferation which is not specifically related to antigenic stimulation. Lymphocytes migrate from these sites to other locations in the body. Germinal centers in other lymphoid tissues, such as lymph nodes and spleen, also actively produce lymphocytes, but do so to a greater extent as a response to antigenic stimulation. Lymphocytes pass through a series of developmental changes in the course of evolving into various lymphocyte

subpopulations, or subsets, yielding a complex interacting system which carries out immune responses. The developmental process organs such as the thymus, where inductive effects on differentiation are mediated via locally produced factors. Mature lymphocytes are engaged in extensive recirculation through the extra vascular and vascular compartments. This is important in facilitating the recognition of foreign antigens by lymphocytes, and it naturally assists the recognition by lymphocytes of foreign antigens to which the individual has been previously exposed. Such immunological memory can persist for many years in circulating lymphocytes which have remained dormant in terms of immunological activity. Cell -mediated and antibody-mediated immune responses involve a complex sequence of events in which lymphocyte subsets interact with other subsets of lymphocytes, as well as the macrophages which play a role in the processing of foreign antigens. The net result of these interactions is the generation of a population of cells with immunological reactivity directed towards the relevant antigen. Mature plasma cells are particularly effectively in antibody production (Firkin *et al.*, 1989).

1.2.1.8. Thrombopoiesis

Platelets are formed in the bone marrow by megakaryocytes, and are subsequently released into the vascular compartment where they play an essential role in haemostasis (Firkin *et al.*, 1989).

1.2.1.9. Regulation of haemopoiesis

1.2.1.9.1. Erythropoiesis

The production of erythropoietin in human increases in response to a reduction in the oxygen –carrying capacity of blood in the descending aorta, and the kidney appears to be the most important organ involved in

this response to reduced tissue oxygen supply. Erythropoietin is a glycoprotein (Firkin *et al.*, 1989).

1.2.1.9.1.1. Erythropoietin

Erythropoietin (EPO), a cytokine is a hormone produced by the kidneys that functions as a targeted erythroid growth factor. This hormone has the ability to stimulate red cell production through a receptor on the pronormoblast, the youngest red cell precursor in the bone marrow. EPO is secreted on a daily basis in small amounts and functions to balance red cell production. If the body becomes anemic and Hgb levels decline, the kidney senses tissue hypoxia and secretes more EPO. Normal red cell maturation from the precursor cell the pronormoblast takes 5 days; with accelerated erythropoiesis, the maturation is decreased to 3 to 4 days. Human recombinant erythropoietin (r-HuEPO) is available product and can be used for individuals experiencing renal disease, for individuals who have become anemic as a result of chemotherapy, or for individuals who refuse whole blood products (Ciesla, 2007).

1.2.1.9.1.2. The cell cycle

The cell division cycle, generally known simply as the cell cycle, is a complex process that lies at the heart of haemopoiesis. Dysregulation of cell proliferation is also the key to the development of malignant disease. The duration of the cell cycle is variable between different tissues but the basic principles remain constant. The cycle is divided into the mitotic phase (Mphase), during which the cell physically divides, and interphase during which the chromosomes are duplicated and cell growth occurs prior to division. The M phase is further partitioned into classical mitosis in which nuclear division is accomplished, and cytokinesis in which cell fission occurs. Interphase is divided into three main stages: a G1 phase in which the cell begins to commit to replication, an S phase during

which DNA content doubles and the chromosomes replicate and the G_2 phase in which the cell organelles are copied and cytoplasmic volume is increased. If cells rest prior to division they enter a Go state where they can remain for long periods of time. The cell cycle is controlled by two checkpoints which act as brakes to coordinate the division process at the end of the G_1 and G_2 phases. Two major classes of molecules control these checkpoints, cyclin dependent protein kinases (Cdk) which phosophorylate down- stream protein targets and cyclins which bind to Cdks and regulate their activity (Hoffbrand *et al*., 2006).

1.2.1.9.1.3. Apoptosis

Apoptosis is a regulated process of physiological cell death in which cells are triggered to activate intracellular proteins that lead to the death of the cell. Morphologically it is characterized by cell shrinkage, condensation of the nuclear chromatin, fragmentation of the nucleus and cleavage of DNA at internucleosomal sites. It is an important process for maintaining tissue homoeostasis in haemopoiesis and lymphocyte development (Hoffbrand *et al*., 2006).

1.2.1.9.1.4. Transcription factors

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families. They contain at least two domains: a DNA-binding domain such as a leucine zipper or helix-loop-helix motif which binds to a specific DNA sequence and an activation domain which contributes to assembly of the transcription complex at a gene promoter (Hoffbrand *et al*., 2006).

1.2.1.9.1.5. Adhesion molecules

A large family of glycoprotein molecules termed adhesion molecules mediates the attachment of marrow precursors, leucocytes and platelets to

various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The adhesion molecules on the surface of leucocytes are termed receptors and these interact with molecules (termed ligands) on the surface of potential target cells (Hoffbrand *et al*., 2006).

1.2.2. Pregnancy

1.2.2.1. Pregnancy trimesters

1.2.2.1.1. Pregnancy first trimester

At the end of the first month, the embryo is about a third of an inch long, and its head and trunk-plus the beginnings of arms and legs-have started to develop. The embryo receives nutrients and eliminates waste through the umbilical cord and placenta. By the end of the first month, the liver and digestive system begin to develop, and the heart starts to beat. Second month in this month, the heart starts to pump and the nervous system begins to develop. The 1 in (2.5 cm) long fetus has a complete cartilage skeleton, which is replaced by bone cells by month's end. Arms, legs and all of the major organs begin to appear. Facial features begin to form. Third month by now, the fetus has grown to 4 in (10 cm) and weighs a little more than an ounce (28 g). Now the major blood vessels and the roof of the mouth are almost completed, as the face starts to take on a more recognizably human appearance. Fingers and toes appear. All the major organs are now beginning to form; the kidneys are now functional and the four chambers of the heart are complete (Williams and Wilkins, 2006).

The haemodilutory effect commences in the first trimester. The lower limit of the hemoglobin level in normal pregnant women is about 10.5 g/dl, and is thus less than in the non-pregnant state (Firkin *et al*., 1989).

1.2.2.1.2. Pregnancy second trimester

Fourth month the fetus begins to kick and swallow, although most women still can't feel the baby move at this point. Now 4 oz (112 g), the fetus can hear and urinate, and has established sleep-wake cycles. All organs are now fully formed, although they will continue to grow for the next five months. The fetus has skin, eyebrows, and hair. Fifth month now weighing up to a 1 lb (454 g) and measuring 8-12 in (20-30 cm), the fetus experiences rapid growth as its internal organs continue to grow. At this point, the mother may feel her baby move, and she can hear the heartbeat with a stethoscope. Sixth month Even though its lungs are not fully developed, a fetus born during this month can survive with intensive care. Weighing 1-1.5 lbs (454-681 g), the fetus is red, wrinkly, and covered with fine hair all over its body. The fetus will grow very fast during this month as its organs continue to develop (Williams and Wilkins 2006).

1.2.2.1.3. Pregnancy third trimester

Seventh month there is a better chance that a fetus born during this month will survive. The fetus continues to grow rapidly, and may weigh as much as 3 lb (1.3 kg) by now. Now the fetus can look around its watery womb with open eyes. Eighth month growth continues but slows down as the baby begins to take up most of the room inside the uterus. Now weighing 4-5 lbs (1.8-2.3 kg) and measuring 16-18 in (40-45 cm) long, the fetus may at this time prepare for delivery next month by moving into the head-down position. Ninth month adding 0.5 lb (227 g) a week as the due date approaches, the fetus drops lower into the mother's abdomen and prepares for the onset of labor, which may begin any time between the 37th and 42nd week of gestation. Most healthy babies will weigh 6-9 lb (2.7-4 kg) at birth, and will be about 20 in. long (Williams and Wilkins 2006).

1.2.2.2. Physiology of pregnancy

Cardiac output increases 30% to 50% in pregnancy. The increase begins at about the sixth week, reaches a maximum about the sixteenth week, declines slightly after the thirtieth week, and rapidly falls off after delivery. It returns to prepregnancy level about the sixth week after delivery. The stroke volume of the heart increases, and the pulse rate becomes more rapid: Normal pulse rate in pregnancy is approximately 80 to 90 beats/min. Blood pressure may drop slightly after the twelfth week of gestation and return to its usual level after the twenty-sixth week. The circulation of blood to the pregnant uterus near term is about 1 L/min, requiring about 20% of the total cardiac output. Total blood volume also increases in pregnancy; plasma volume increases more than red cell volume, and these results in a drop in the hematocrit, caused by dilution. The number of white blood cells increases: The normal white blood cell count in pregnancy is often above 15,000/µl (Williams and Wilkins, 2006).

Physiological variation in the blood count occurs during pregnancy, the Hb falls, the MCV rises slightly and the WBC and neutrophil count rise. Immature cells (myelocytes and occasional promyelocytes) appear in the blood and there may be ‘toxic 'granulation and Dohle bodies (Bain, 1996).

1.2.2.3. Hematological changes associated with pregnancy

1.2.2.3.1. Erythropoietic activity

In normal pregnancy, there is an increase in erythropoietic activity. However, at the same time, an increase in plasma volume occurs, and this results in a progressive decrease in Hb, Hct and RBC. There is a slight increase in MCV during the 2nd trimester. Serum ferritin decreases in

early pregnancy and usually remains low throughout pregnancy, even when supplementary iron is given (Dacie and Lewis, 2011).

1.2.2.3.2. White blood cells

WBC and neutrophil count rise. Immature cells (myelocytes and occasional promyelocytes) appear in the blood and there may be ‘toxic 'granulation and Dohle bodies (Bain, 1996).

1.2.2.3.3. Iron status

In early pregnancy serum ferritin concentrations usually provide a reliable indication of iron deficiency. Haemodilution in the 2nd and 3rd trimesters of pregnancy reduces the concentrations of all measures of iron status and this means that the threshold values for iron deficiency established in non-pregnant women are not appropriate. In principle, determination of values as ratios (ZPP mmol/mol haem, transferrin saturation and sTfR/ferritin) should be more reliable. In healthy women who were not anemic and who were supplemented with iron, serum iron, transferrin saturation and serum ferritin fell from the 1st to the 3rd trimester, TIBC increased during pregnancy. sTfR concentrations showed a substantial increase (approximately two-fold) during pregnancy and this probably reflects increased erythropoiesis (Dacie and Lewis, 2011).

1.2.3. Anemia

Defined as a reduction in the hemoglobin concentration less than 13.5 g/dl in adult males, less than 11.5 g/dl in adult females, and less than 14.0 g/dl in newborn infants. Reduction of hemoglobin is usually accompanied by a fall in red cell count and packed cell volume (PCV). An increase in plasma volume (as with splenomegaly or pregnancy) may cause anemia even with a normal total circulating red cell and hemoglobin mass (Hoffbrand *et al*., 2006).

1.2.3.1. Symptoms and signs of anemia

If the patient does have symptoms these are usually shortness of breath particularly on' exercise, weakness, lethargy, palpitation and headaches. In older subjects, symptoms of cardiac failure, angina pectoris or intermittent claudication or confusion may be present. Visual disturbances because of retinal hemorrhages may complicate very severe anemia, particularly of rapid onset. General signs include pallor of mucous membranes which occurs if the hemoglobin level is less than9-10g/dl conversely, skin color is not a reliable sign. A hyper dynamic circulation may be present with tachycardia, a bounding pulse, cardiomegaly and a systolic flow murmur especially at the apex. Particularly in the elderly, features of congestive heart failure may be present. Specific signs are associated with particular types of anemia (e.g. koilonychias 'spoon nails' with iron deficiency, jaundice with hemolytic or megaloblastic anaemias (Hoffbrand *et al.*, 2006).

1.2.3.2. Classification of anemia

Classifications group of anemias based on erythrocyte morphology, physiology, or probable etiology. The method based on red cell morphology, which was categorizes anemias by the size of the erythrocytes as macrocytic, normocytic, or microcytic. A classification system that divides the major pathophysiological characteristics into three major categories, accelerated erythrocyte destruction, blood loss, and impaired RBC production. (Turgeon, 2010).

1.2.3.3. Physiological adaptations in anemia

Tissue hypoxia develops when compensatory physiological adjustments that enhance release of oxygen from hemoglobin, and increase the flow of blood to the tissues, fail to counteract the effects of the decreased oxygen-

carrying capacity of the blood. Hypoxia causes impairment of function in many tissues, and the symptoms and signs of anemia are therefore referred to many systems with high requirements, such as the skeletal musculature during activity, the heart and the central nervous system, are particularly prominent. Several mechanisms are brought into play in anemia to make more effective use of the available hemoglobin for delivery of oxygen to the tissues. Increased release of oxygen from red cells. This results from the increase in concentration of 2, 3-di - phosphoglycerate which takes place in the red cell in anemia, the oxygen dissociation curve is shifted to the right. Also increased blood flow cardiac output in anemia (Firkin *et al.*, 1989).

1.2.3.4. Laboratory assessment of anaemias

Laboratory investigation of anemia involves the quantitative (Hb, PCV, RBCs count and indices, RDW), and semi quantitative measurements of erythrocytes morphology and supplementary testing of blood and body fluids (Turgeon, 2010).

1.2.3.5. Common anemia associated with pregnancy

1.2.3.5.1. Iron deficiency Anemia

Iron deficiency is the most common cause of anemia in pregnancy. The majority of pregnant women with hemoglobin values of less than 10 g/ dl are suffering from iron deficiency anemia, although frequently there is definite iron deficiency in patients with hemoglobin values above this figure. The demands of previous pregnancies render women especially prone to iron deficiency, particularly when the interval between pregnancies is short. It is not uncommon for multiparous women or women with heavy menstrual loss to become pregnant with either pre-existing iron deficiency anemia or no Iron stores (Firkin *et al.*, 1989).

1.2.3.5.1.1. Distribution of body iron

The concentration of iron in the adult human body is normally about 50 mg/kg in males and 40 mg/kg in females. The largest component is circulating hemoglobin; with 450 ml (1 unit) of whole blood containing about 200 mg of iron. Much of the remainder is contained in the storage proteins, ferritin and haemosiderin. These are found mainly in the reticuloendothelial (RE) cells of the liver, spleen and bone marrow (which gain iron from breaking down red cells), and in parenchymal liver cells (which normally gain most of their iron from the plasma iron-transporting protein, transferrin) (Hoffbrand *et al.*, 2005).

1.2.3.5.1.2. Dietary iron

Iron is present in food as ferric hydroxides, ferric protein and haem-protein complexes. Both the iron content and the proportion of iron absorbed differ from food to food; in general, meat-in particular liver-is a better source than vegetables, eggs or dairy foods, The average Western diet contains 10-15 mg iron daily from which only 5-10% is normally absorbed, The proportion can be increased to20-30% in iron deficiency or pregnancy (Hoffbrand *et al*., 2006).

1.2.3.5.1.3. Plasma (transport) iron

Between 3 and 4 mg iron are present in the plasma, where it is bound to a specific protein, transferrin, which is synthesized in the liver. Each molecule of transferring binds one or two atoms of ferric iron. The function of transferrin is the transport of iron. It is the means by which iron absorbed from the alimentary tract is transported to the tissue stores from tissue stores to bone marrow erythroblasts, and from one storage site to another. When transferrin reaches the storage sites or the bone marrow, it attaches to specific receptors on cells and liberates its ferric ions, which

pass into the cell to be stored or utilized. Transferrin receptors have been demonstrated on reticulocytes and erythroblasts. Plasma iron is continually being recycled with a turnover time of approximately three hours. The total amount of transferrin in the plasma is about 8 g, and a similar amount (binding 3-4 mg iron) is in the extracellular fluid, in equilibrium with plasma transferrin (Firkin *et al*., 1989).

1.2.3.5.1.4. Iron absorption

Organic dietary iron is partly absorbed as haem and partly broken down in the gut to inorganic iron. Absorption occurs through the duodenum. Haem is absorbed through a specific receptor, HCP-I, exposed on the apical membrane of the duodenal enterocyte. Haem is then digested to release iron.

The protein DMT-I (divalent metal transporter) is involved in transfer of iron. The amount of iron absorbed is partly regulated according to the body's needs by changing the levels of DMT-l according to the iron status of the duodenal villous crypt enterocyte. In iron deficiency less iron is delivered to the crypt cell from transferring which is largely unsaturated with iron. Hepcidin is also a major regulator by affecting ferroportin concentration. Low hepcidin levels in iron deficiency increase ferroportin levels and allow more iron to enter portal plasma (Hoffbrand *et al.*, 2006).

1.2.3.5.1.5. Iron requirements

The amount of iron required each day to compensate for losses from the body and for growth varies with age and sex; it is highest in pregnancy, adolescent and menstruating females'. Therefore these groups are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake (Hoffbrand *et al.*, 2006).

1.2.3.5.1.6. Factors favoring absorption

Haem iron, Ferrous form (Fe2+),Acids (HCl, vitamin C), solubilizing agents (e.g. sugars, amino acids),Iron deficiency, Ineffective erythropoiesis, Pregnancy, Hereditary haemochromatosis Increased expression of DMT-1 and ferroportin. in duodenal enterocytes (Hoffbrand *et al.*, 2006).

1.2.3.5.1.7. Factors reducing absorption

Inorganic iron, Ferric form (Fe^{3+}),alkalis-antacids, pancreatic secretions, precipitating agents-phytates, phosphates, iron excess, decreased erythropoiesis, infection, tea, and decreased expression of DMT-1 and ferroportin in duodenal enterocytes Increased hepcidin (Hoffbrand *et al.*, 2006).

1.2.3.5.1.8. Clinical features of iron deficiency anemia

When iron deficiency is developing the reticuloendothelial stores (haemosiderin and ferritin) become completely depleted before anemia occurs. As the condition develops the patient may develop the general symptoms and signs of anemia and also show a painless glossitis, angular stomatitis, brittle, ridged or spoon nails (koilonychia), dysphagia as a result of pharyngeal webs and unusual dietary cravings (pica). The cause of the epithelial cell changes is not clear but may be related to reduction of iron in iron-containing enzymes. In children, iron deficiency is particularly significant as it can cause irritability, poor cognitive function and a decline in psychomotor development (Hoffbrand *et al.*, 2006). .

1.2.3.5.1.9. Causes of iron deficiency

Chronic blood loss, especially uterine or from the gastrointestinal tract, is the dominant cause. In contrast, in developed countries dietary deficiency is rarely a cause on its own. Half a liter of whole blood contains

approximately 250 mg of iron and, despite the increased absorption of food iron at an early stage of iron deficiency, negative iron balance is usual in chronic blood loss. Increased demands during infancy, adolescence, pregnancy, lactation and in menstruating women account for the high risk of anemia in these particular clinical groups. Newborn infants have a store of iron derived from delayed clamping of the cord and the breakdown of excess red cells. From 3 to 6 months there is a tendency for negative iron balance because of growth. From 6 months supplemented formula milk and mixed feeding, particularly with iron-fortified foods, prevents iron deficiency. In pregnancy increased iron is needed for an increased maternal red cell mass of approximately35%, transfer of 300 mg of iron to the fetus and because of blood loss at delivery. Although iron absorption is also increased, iron therapy is often needed if the hemoglobin (Hb) falls below 10 g/dl or the mean cell volume (MCV) is below 82 fl in the third trimester. Menorrhagia (a loss of 80 ml or more of blood at each cycle) is difficult to assess clinically, although the loss of clots, the use of large numbers of pads or tampons or prolonged periods all suggest excessive loss. In clinical practice inadequate intake or malabsorption are only rarely the sole causes of iron deficiency anemia although in developing countries iron deficiency may occur as a result of a life-long poor diet, consisting mainly of cereals and vegetables. Gluten-induced enteropathy, partial or total gastrectomy and atrophic gastritis (often autoimmune and with Helicobacter pylori infection) may, however, predispose to iron deficiency (Hoffbrand *et al.*, 2006).

1.2.3.5.1.10. Laboratory findings in iron deficiency anemia

The red cell indices fall. The blood film shows hypochromic microcytic cells with occasional target cells and pencil-shaped poikilocytes. The

reticulocyte count it is low in relation to the degree of anemia. When iron deficiency is associated with severe folate or vitamin B_{12} deficiency a 'dimorphic' film occurs with a dual population of red cells of which one is macrocytic and the other microcytic and hypochromic; the indices may be normal. A dimorphic blood film is also seen in patients with iron deficiency anemia who have received recent iron therapy and produced a population of new haemoglobinized normal-sized red cells and when the patient has been transfused. The platelet count 1t is often moderately raised in iron deficiency, particularly when hemorrhage is continuing (Hoffbrand *et al.*, 2006).

1.2.3.5.1.11 Sequence of events of Iron deficiency anemia

1.2.3.5.1.11.1. Depletion of iron stores

When the body is in a state of negative iron balance, the first event is depletion of body stores, which are mobilized for hemoglobin production (Hoffbrand *et al*., 2005).

1.2.3.5.1.11.2. Iron-deficient erythropoiesis

With further iron depletion, when the serum ferritin is below150 g/L, the serum transferrin saturation falls to less than 15% due to a rise in transferrin concentration and a fall in serum iron. This leads to the development of iron-deficient erythropoiesis and increasing concentrations of serum transferrin receptor and red cell protoporphyrin. At this stage, the Hb, MCV, and MCH, may still be within the reference range, although they may rise significantly when iron therapy is given (Hoffbrand *et al*., 2005).

1.2.3.5.1.11.3. Iron deficiency anemia

The red cells become obviously microcytic and hypochromic and poikilocytosis becomes more marked. The MCV and MCH are reduced,

and target cells may be present. The reticulocyte count is low. The serum TIBC rises and the serum iron falls, so that the percentage saturation of the TIBC is usually less than 10%.The number of erythroblasts containing cytoplasmic iron (sideroblasts) is reduced at an early stage in the development of deficiency, and siderotic granules are entirely absent from these cells when iron deficiency anemia is established. The erythroblasts have a ragged, vacuolated cytoplasm and relatively pyknotic nuclei. Platelets are frequently increased (Hoffbrand *et al*., 2005).

1.2.3.5.1.11.4. Tissue effects of iron deficiency

When iron deficiency is severe and chronic, widespread tissue changes may be present, including koilonychias (ridged nails, breaking easily), angular stomatitis, glossitis (hair thinning) and pharyngeal webs (Paterson–Kelly syndrome). There is recent evidence that premature labour is more frequent in mothers with iron deficiency anemia (Hoffbrand *et al*.,2005).

1.2.3.5.1.12. Management of iron deficiency

Management entails (i) identification and treatment of the underlying cause and (ii) correction of the deficiency by therapy with inorganic iron. Iron deficiency is commonly due to blood loss and, wherever possible, the site of this must be identified and the lesion treated (Hoffbrand *et al*., 2005).

1.2.3.5.2. Megaloblastic anaemias

This is a group of anaemias in which the erythroblasts in the bone marrow show a characteristic abnormality-maturation of the nucleus being delayed relative to that of the cytoplasm. The underlying defect accounting for the asynchronous maturation of the nucleus is defective DNA synthesis (Hoffbrand *et al*., 2006).

1.2.3.5.2.1. Vitamin B12, cobalamin absorption and Transport

This vitamin is synthesized in nature by microorganisms; animals acquire it by eating other animal foods, by internal production from intestinal bacteria (not in humans) or by eating bacterially contaminated foods. The vitamin is found in foods of animal origin such as liver, meat, fish and dairy produce. A normal diet contains a large excess of B12 compared with daily needs. Bl2 is combined with the glycoprotein intrinsic factor (IF) which is synthesized by the gastric parietal cells. The IF-B12 complex can then bind to a specific surface receptor for IF, cubilin, which then binds to a second protein, amnionless which directs endocytosis of the cubilin IF-B12 complex in the distal ileum where Bl2 is absorbed and IF destroyed. Vitamin Bl2 is absorbed into portal blood where it becomes attached to the plasma-binding protein transcobalamin (TC, previously called transcobalaminII) which delivers Bl2 to bone marrow and other tissues. Although TC is the essential plasma protein for transferring Bl2 into the cells of the body, the amount of Bl2 on TC is normally very low<50 ng/L). TC deficiency causes megaloblastic anemia because of failure of B12 to enter marrow (and other cells) from plasma but the serum B12.level in TC deficiency is normal. This is because most B12 in plasma is bound to another transport protein, haptocorrin (previously called transcobalamin I).This is a glycoprotein largely synthesized by granulocytes and macrophages. In myeloproliferative diseases where granulocyte production is greatly increased, the haptocorrin and B12 levels in serum both rise considerably. B12 bound to haptocorrin does not transfer to marrow; it appears to be functionally 'dead'. Closely related glycoproteins to plasma haptocorrin are present in gastric juice, milk and other body fluids (Hoffbrand *et al*., 2006).

1.2.3.5.2.2. Folate absorption, transport and function

Folic (pteroylglutamic) acid is the parent compound of a large group of compounds, the folates, that are derived from it.

Humans are unable to synthesize the folate structure and thus require preformed folate as a vitamin. Dietary folates are converted to methyl THF (which, like folic acid, contains only one glutamate moiety) during absorption through the upper small intestine. Once inside the cell they are converted to folatepolyglutamates. Folate binding proteins are present on cell surfaces including the enterocyte and facilitate entry of reduced folates into cells. There is no specific plasma protein that enhances cellular folate uptake. Folates are needed in a variety of biochemical reactions in the body involving single carbon unit transfer, in amino acid interconversions (e.g. homocysteine conversion to methionine) and serine to glycine or in synthesis of purine precursors of DNA (Hoffbrand *et al.*, 2006).

1.2.3.5.2.3. Megaloblastic anemia of pregnancy

Megaloblastic anemia during pregnancy results from an inadequate intake of folate to meet the increased requirements of pregnancy. A small proportion of cases are due to latent coeliac disease first becoming manifest during pregnancy. Rare cases are due to the fortuitous association of pernicious anemia; although this disorder is uncommon in the child-bearing age group. The prevalence of megaloblastic anemia of pregnancy varies in different populations, apparently depending on the nutritional status of the population. In well-nourished communities, florid forms are now rare, but mild cases occasionally occur in spite of the widespread use of prophylactic folic acid (Firkin *et al.*, 1989).

1.2.3.5.2.3.1. Pathogenesis of megaloblastic anemia of pregnancy

Folate is required by the fetus for normal development, and an adequate supply is assured at the expense of the mother. In normal pregnancy, the average folate requirement is increased three-fold. There is a progressive fall in serum folate values, subnormal levels occurring in about 50 per cent of patients in the last trimester. Reduction in the red cell folate level is less frequent. These changes are not necessarily accompanied by anemia or abnormalities in the blood or bone marrow. If pre-existing folate deficiency is present, or the dietary folate intake of the mother is inadequate to meet the increased demand; tissue deficiency of folate occurs and megaloblastic changes become evident in the bone marrow. Mild bone marrow changes, not necessarily associated with anemia, are seen in 20-30 per cent of pregnant women in late pregnancy. In the occasional case, further progression to a frank megaloblastic anemia occurs. Other factors, besides fetal demand, that may contribute to the development of anemia include iron deficiency, co-existent hemolytic anemia, urinary tract and other infections, anticonvulsant and trimethoprim therapy, and altered intestinal absorption of folate (Firkin *et al*., 1989).

1.2.3.5.2.3.2. Clinical features of megaloblastic anemia of pregnancy

Megaloblastic anemia of pregnancy tends to occur more frequently after multiple pregnancies than in first and second pregnancies. Onset is usually gradual in late pregnancy, but may be rapid, particularly when associated with the presence of infection. Anorexia, excessive vomiting, and moderate weight loss are common, and glossitis and diarrhea are features in some cases. Breast milk contains folate and occasional cases occurring during prolonged lactation in a poorly nourished mother. Spontaneous remission following delivery is usual, even in the absence of

treatment. With early diagnosis and adequate treatment, the outlook for mother and child is good (Firkin *et al*., 1989).

1.2.3.5.2.3.3. Diagnosis of megaloblastic anemia of pregnancy

The degree of anemia and abnormalities of red cell morphology vary. Frequently, the blood picture is similar to that of pernicious anemia, with marked oval macrocytosis. However, in some cases these features are much less marked, and the anemia may be normocytic rather 'than macrocytic and the MCV within normal range. Not uncommonly there is a concomitant iron deficiency, and the film is that of a 'dimorphic' anemia and hypersegmented neutrophils. Megaloblastic anemia of pregnancy, although relatively uncommon, should be considered in any pregnant patient who is anemic without obvious cause, especially in the third trimester or puerperium. Although there is a natural reluctance to perform bone marrow aspiration in late pregnancy, this examination is essential to establish a definitive diagnosis, as the levels of serum and red cell folate are often reduced at term in normal pregnancy and are not of great help. In some patients, the serum vitamin B_{12} is also subnormal, but the level usually returns to normal after folic acid therapy even though vitamin B_{12} is not given (Firkin *et al*., 1989).

1.2.3.5.2.3.4. Prevention of megaloblastic anemias of pregnancy

Prophylactic administration of folic acid as well as iron during pregnancy. The daily supplement usually recommended is 300 µg. A number of proprietary tablets containing both iron and folic acid are available; combined preparations have the advantage that the patient need take only one tablet a day. (Firkin *et al*., 1989).

1.2.4. Previous study

1.2.4.1. Previous study in West Bengal, India

In previous study of hematological parameters in pregnancy the results showed that study group exhibited statistically significant lower values of hemoglobin, PCV of pregnant women compared with the control ($p< 0.05$). while WBC were significantly elevated. There was no significant difference in all hematological parameters among the three trimesters. The value of neutrophil is higher in the studied group than the control group, but there is no statistical difference between the value of neutrophil in both the study and control groups. In this present study lymphocyte counts were lower in studied group than in control. Comparison of Hematological indices in pregnant women and control (Mean ± SD), Hb (gm/dl) 9 ± 1.5, 12.0 ± 0.6, P.C.V (%) 30.68 ± 4.26, 37.73 ± 3.69, WBC (x 10^9/L) 7.26 ± 3.02, 4.91 ± 0.88 Neutrophil (%)51.89 ± 13.88, 43.61 ± 0.87, Lymphocyte (%)34.68 ± 14.52, 43.84 ± 12.52, $p \leq 0.05$ comparing with the control group. When grouped by trimesters, the mean value± standard deviation at third trimester of Hb gm/dl 9 ± 1.4, PCV (%) 31.58 ± 5.48, WBC (x10^9/L) 8.09 ± 4.12, Neutrophil(%)55.31 ± 11.97, Lymphocyte (%)32.68 ± 12.51 (Das S *et al.*, 2013).

1.2.4.2. Previous study in Nigeria

In previous study of hematological profile of normal pregnant women in Lagos, Nigeria they were found that (mean ± standard deviation): hematocrit 30.16 % ± 5.55 % ; hemoglobin 10.94 ± 1.86 g/dl; white blood cells, 7.81 ± 2.34 ×10^9/L; platelets, 228.29 ±65.6×10^9/L; cell volume 78.30 ±5.70 fl, corpuscular hemoglobin, 28.57 ±2.48 pg; and corpuscular hemoglobin concentration, 36.45 ±1.10 g/dl. When grouped by trimesters, the mean value± standard deviation at third trimester of

packed cell volume, 33.04 % ±3.88%, hemoglobin 10.38± 1.72 g/dl, white blood cell concentration 8.37 ±2.15 $\times 10^9$/L, and platelet values were 200.82 ±94.42 $\times 10^9$/L. A statistically significant relationship was found to exist between packed cell volume and white blood cell count with increase in gestational age (P = 0.010 and 0.001,respectively). However, there was no statistically significant association between platelet count and increase in gestational age (P = 0.296) (Akinbami *et al.*, 2013).

1.2.4.3. Previous study in Sudan

In previous study, assessment of complete blood count of Sudanese pregnant women in Port Sudan city by Khalil and Rania, publishing by Sudan University of Science and Technology (2012). The results indicated that WBC (mean =8.0×10^3/µl±2.1) of pregnant women with number of pregnancy between (1-3)pregnancies increased insignificantly(p.value> 0.05) compared to those of pregnancy between(4-7) and (7-10).WBC(mean =8.0×10^3/µl±2.1) of pregnant women at third trimester increased insignificantly (p.value 0.08)while lymphocytes(mean=25%±7.0) decreased significantly (p.value 0.01) than those women at first and second trimesters Neutrophils (mean=66.2%± 8.0) of women at third trimester increased significantly compared to those in first and second trimesters (p.value 0.02),when compared of different trimesters with control the result was WBCs and neutrophils increased significantly at third trimester (p.value 0.00) but for lymphocytes was decreased significantly at third trimester (p,value 0,00)MPV(mean =10.1 fl±1.0) of pregnant women with history of abortion significantly increased compared to those with no history of abortion (p.value 0.03).RBC(mean =4.2×10^6/µl± 0.5) and HCT (mean =34.5%±3.6) of

pregnant women increased significantly (p.value0.01) with regularly visits to clinics (Khalil, 2012).

1.3. Rationale

The rationale for conducting this research in pregnant women at third trimester is to advance knowledge in the medical conditions in pregnant women, prenatal conditions that might threaten the health of the fetus, physiologic changes that accompany pregnancy, and medical conditions related to pregnancy that might affect the future health of women.

1.4. Objectives

1.4.1. General objective

To measure complete blood cell count of Sudanese pregnant women in the third trimester - Khartoum locality.

1.4.2. Specific objectives

- To count red blood cells and its indices, white blood cells and its differential, and platelets and its indices in test and control groups.

- To compare between means red blood cells count and its indices, white blood cells count and its differential, and platelets count and its indices in test and control groups.

-To correlate between regular visit to clinic, abortion, age, numbers of children and supplemental drugs used on pregnant women third trimester.

Chapter Two

Materials and Methods

2.1. Study design:

This study is case control study conducted in period from January to March 2015 to measure CBC in pregnant women at third trimester.

2.2. Study population:

Study carried out in 120 Sudanese women 80 as study group and 40 non pregnant as control in Khartoum locality.

2.3. Inclusion criteria:

Healthy pregnant women in the third trimester, and all age groups were included.

2.4. Exclusion criteria:

Presence of any diagnostic diseases such as anemia, previous blood transfusion, and typhoid were excluded.

2.5. Ethical consideration:

An informed consent from selected individuals to be study was taken after being informed with all detailed objective of the study.

2.6. Data collection:

Data was collected using questionnaire which was specifically designed to obtain information about demographic and clinical data that helped in either including or excluding certain individual in or from the study respectively.

2.7. Sample collection

2.5 ml venous blood was collected from individual under study and dispensed in EDTA container for CBC (Dacie and Lewis, 2011).

2.8. Procedure of complete blood count (CBC)

Fully automated multichannel instruments require only that an appropriate blood sample is presented to the instrument and usually measure from 8 to 20 components for the basic CBC and white blood cell differential. Impedance counting systems depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors. (Dacie and Lewis, 2011)

2.8.1. Hemoglobin concentration (HGB or HB)

Automated counter used nonhazardous chemical, such as sodium lauryl sulphate, imidazole, and sodium dodecyl sulphate or dimethyl laurylamineoxide. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A detergent is included to ensure rapid cell lysis and to reduce turbidity. Measurements of absorbance are made for hemoglobin measurement at various wavelengths depending on the final stable haemochromogen, cyanmethaemoglobin, oxyhaemoglobin, methaemoglobin or monohydroxyferri-porphyrin. Hemoglobin concentration values, in normal female 135 ± 15 g/L, in pregnancy 1st trimester 124–135 g/l, 2nd trimester 110–117 g/l, and 3rd trimester 106–109 g/l, 120 g/l or higher may be found when supplementary iron is being given (Dacie and Lewis, 2011).

2.8.2. Red blood cell count (RBC) and Platelet count

Red cells and other blood cells were counted in systems based on aperture impedance technology. Platelets can be counted in whole blood using the

same techniques of electrical detection as is used for counting red cells. An upper threshold is needed to separate platelets from red cells and a lower threshold is needed to separate platelets from debris and electronic noise. RBC normal range in women $4.3 \pm 0.5 \times 10^{12}$/L, and platelet normal range in women $280 \pm 130 \times 10^{9}$/L (Dacie and Lewis, 2011).

2.8.3. Packed cell volume (PCV)

Automated blood cell counter was estimated PCV/haematocrit by technology that has little connection with packing red cells by centrifugation. The passage of a cell through the aperture of an impedance counter leads to the generation of an electrical pulse, the of which is proportional to cell volume. The number of pulses generated allows the RBC to be determined. Women normal range 0.41 ± 0.05 L/L (Dacie and Lewis, 2011).

2.8.4. Red cell indices

2.8.4.1. Mean cell volume (MCV)

MCV is measured directly. Women normal range 92 ± 9 fl (Dacie and Lewis, 2011).

2.8.4.2. Mean corpuscular hemoglobin (MCH)

The mean amount of hemoglobin per red cell (MCH) is reliably estimated by automated electronic counting devices by dividing the total amount of hemoglobin by the number of red cells in a sample of blood. Women normal range 29.5 ±2.5 pg (Firkin *et al*., 1989).

2.8.4.3. Mean cell hemoglobin concentration (MCHC)

The MCHC is derived in the traditional manner from the Hb and the Hct with instruments that measure the Hct and calculate the MCV.

MCHC=Hb / Hct×100. Women normal range 330 ±15 g/L (Dacie and Lewis, 2011).

2.8.4.4. Red cell distribution width (RDW)

The RDW is derived from pulse height analysis and can be expressed either as the standard deviation (SD) in fl or as the coefficient of variation (CV) (%) of the measurements of the red cell volume. The RDW SD is measured by calculating the width in fl at the 20% height level of the red cell size distribution histogram and the RDW CV is calculated mathematically as the coefficient of variation, i.e. RDW (CV) % ¼ 1SD/MCV _ 100%. The normal reference range is in the order of 12.8 ±1.2% as CV and 42.5 ±3.5 fl as SD. (Dacie and Lewis, 2011).

2.8.5. Total white blood cell count (WBC)

The WBC is determined in whole blood in which red cells have been lysed. The lytic agent is required to destroy the red cells and reduce the red cell stroma to a residue that causes no detectable response in the counting system. The following fluid is satisfactory: Cetrimide 20 g,10% formaldehyde (in 9 g/l NaCl) 2 ml, Glacial acetic acid 16 ml, NaCl 6 g, and water to 1 liter. Residual particles in a diluted blood sample are counted after red cell lysis. Normal range 4 - 10 × 10^9 L (Dacie and Lewis, 2011).

2.8.6. Automated differential count

Automated blood cell counter have a differential counting capacity, providing a three-part differential count. Counts are performed on diluted whole blood in which red cells are either lysed or are rendered transparent. A three-part differential count was categorized leucocytes as WBC-small cell ratio (equivalent to lymphocytes), WBC-middle cell ratio (equivalent to monocytes, eosinophils and basophils) and WBC-

large cell ratio (equivalent to neutrophils). Normal differential count neutrophils 40- 80 %, lymphocytes 20-40 %, monocytes 2-10 %, eosinophils 1-6 %, basophils < 1-2% (Dacie and Lewis, 2011). ..

2.8.7. Mean platelet volume and platelet distribution width

The mean platelet volume (MPV) is derived from the impedance platelet size distribution curve. The MPV is lower than predicted when thrombocytopenia is caused by megaloblastic anemia or bone marrow failure an increase in MPV has been observed in patients at risk of and following myocardial infarction and cerebral infarction. Other platelet parameters platelet distribution width (PDW), which is a measure of platelet anisocytosis and the 'plateletcrit', which is the product of the MPV and platelet count and, by analogy with the haematocrit, may be seen as indicative of the volume of circulating platelets in a unit volume of blood. A high PDW may indicate peripheral immune destruction of platelets. MPV is the average volume of individual platelets derived from the Plt histogram. It represents the mean volume of the Plt population under the fitted Plt curve multiplied by a calibration constant, and expressed in femtoliters (Dacie and Lewis, 2011).

2.9. Data analysis:

Data were entered into computer and analyzed by SPSS used independent test and one way ANOVA test.

Chapter Three

Results

-There was significant decreased in mean of Hct, TRBCs, RDWSD, platelets count and lymphocytes percentage and absolute count in pregnant women when compared with control and, significant increased in means of MCHC, TWBCs and neutrophils percentage and absolute of pregnant women more than control.Insignificant decreased in means of Hb, MCV, RDWCV and PDW in pregnant women when compared with control. Insignificant increased in means of MCH and MPV of pregnant women more than control (tables 3.1, 3.2, and 3.3).

-There was significant increased in means of MPV of with abortion when compared to without abortion. No significant difference in means of Hb, Hct, TRBCs count and indices and TWBCs count, differential and absolute between with and without abortion (tables 3.4,3.5, and 3.6).

-There was significant increased in means of Hct, MCV, and RDWSD in < 2 more than >2 children. No significant difference in means of Hb, TRBCs, MCH, MCHC, RDWCV, TWBCs count, differential and absolute and platelet count and indices between < 2 and >2 children(tables 3.7,3.8, and 3.9).

-There was significant increased in means of Hct, MCV, and MCH in < 30 more than > 30 years. No significant difference in means of Hb, TRBCs, MCHC, RDWSD, RDWCV, TWBCs count, differential and absolute, and platelet count and indices between < 30 and > 30 years(tables 3.10,3.11, and 3.12).

Table (3.1) Comparison between pregnant women and control (Hb, Hct,RBCs count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Hb g/dl	Pregnant	80	11.8	1.19	0.33
	Control	40	12.0	1.19	
Hct %	Pregnant	80	35.2	3.00	0.00
	Control	40	38.7	3.55	
TRBCs $\times 10^{12}$ /l	Pregnant	80	4.1	0.35	0.00
	Control	40	4.5	0.45	
MCV fl	Pregnant	80	84.1	5.62	0.43
	Control	40	85.2	8.07	
MCH pg	Pregnant	80	27.8	3.73	0.80
	Control	40	26.6	2.65	
MCHC %	Pregnant	80	33.4	1.48	0.00
	Control	40	31.0	1.33	
RDWSD	Pregnant	80	44.3	3.90	0.01
	Control	40	46.6	6.13	
RDWCV	Pregnant	80	14.2	1.31	0.69
	Control	40	14.3	1.29	

Table (3.2) Comparison between pregnant women and control (TWBCs count, differential and absolute)

Test	Sample	No	Mean	Std. deviation	P value
TWBCs $\times 10^9$ /l	Pregnant	80	7.4	2.44	0.00
	Control	40	5.9	1.63	
Lymph %	Pregnant	80	26.6	9.90	0.00
	Control	40	38.9	8.01	
Neutr %	Pregnant	80	63.4	11.31	0.00
	Control	40	49.1	11.89	
Lymph $\times 10^9$ /l	Pregnant	80	1.8	0.66	0.00
	Control	40	2.2	0.46	
Neutr $\times 10^9$ /l	Pregnant	80	4.8	2.19	0.00
	Control	40	3.0	1.41	

Table (3.3) Comparison between pregnant women and control (platelet count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Plt $\times 10^9$ /l	Pregnant	80	212.0	64.50	0.00
	Control	40	289.5	79.74	
PDW	Pregnant	80	14.9	2.19	0.38
	Control	40	15.2	0.82	
MPV	Pregnant	80	9.5	1.60	0.48
	Control	40	9.3	1.21	

Table (3.4) Comparison between pregnant women with and without abortion (Hb, Hct, RBCs count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Hb g/dl	yes	47	12.0	1.32	0.22
	no	33	11.7	1.13	
Hct %	yes	47	35.8	3.17	0.21
	no	33	34.9	2.90	
TRBCs $\times 10^{12}$ /l	yes	47	4.2	0.33	0.44
	no	33	4.1	0.36	
MCV fl	yes	47	84.5	5.39	0.65
	no	33	83.9	5.76	
MCH pg	yes	47	27.2	5.65	0.43
	no	33	28.1	2.42	
MCHC %	yes	47	33.4	1.72	0.89
	no	33	33.4	1.37	
RDWSD	Yes	25	45.0	3.78	0.32
	No	55	44.0	3.95	
RDWCV	yes	25	14.3	1.21	0.71
	no	55	14.2	1.37	

Table (3.5) Comparison between pregnant women with and without abortion (TWBCs count, differential and absolute)

Test	Sample	No	Mean	Std. deviation	P value
TWBCs $\times 10^9$ /l	yes	47	7.8	2.46	0.42
	no	33	7.3	2.44	
Lymph %	yes	47	25.5	7.77	0.52
	no	33	27.1	10.76	
Neutr %	yes	47	63.1	11.59	0.84
	no	33	63.6	11.29	
Lymph $\times 10^9$ /l	yes	47	1.8	0.62	0.94
	no	33	1.8	0.69	
Neutr $\times 10^9$ /l	yes	47	5.1	2.26	0.45
	no	33	4.7	2.11	

Table (3.6) Comparison between pregnant women with and without abortion (platelet count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Plt $\times 10^9$ /l	yes	47	201.7	69.08	0.33
	no	33	216.7	62.40	
PDW	yes	25	15.3	2.28	0.35
	no	55	14.8	2.15	
MPV	yes	25	10.2	1.65	0.01
	no	55	9.2	1.51	

Table (3.7) Comparison between pregnant women according to numbers of children (Hb, Hct, RBCs count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Hb g/dl	<2	43	11.9	0.99	0.16
	>2	37	11.6	1.38	
Hct %	<2	43	35.9	2.56	0.01
	>2	37	34.3	3.25	
TRBCs $\times 10^{12}$ /l	<2	43	4.1	0.33	0.42
	>2	37	4.2	0.37	
MCV fl	<2	43	85.6	5.30	0.01
	>2	37	82.4	5.55	
MCH pg	<2	43	28.4	2.40	0.10
	>2	37	27.1	4.77	
MCHC %	<2	43	33.2	1.29	0.13
	>2	37	33.7	1.64	
RDWSD	<2	43	45.3	4.02	0.01
	>2	37	43.2	3.45	
RDWCV	<2	43	14.3	1.37	0.81
	>2	37	14.2	1.26	

Table (3.8) Comparison between pregnant women according to numbers of children (TWBCs count, differential and absolute)

Test	Sample	No	Mean	Std. deviation	P value
TWBCs $\times 10^9$ /l	<2	43	7.6	2.47	0.39
	>2	37	7.2	2.42	
Lymph %	<2	43	26.8	11.05	0.85
	>2	37	26.3	8.52	
Neutr %	<2	43	63.8	11.59	0.76
	>2	37	63.0	11.12	
Lymph $\times 10^9$ /l	<2	43	1.9	0.67	0.37
	>2	37	1.8	0.65	
Neutr $\times 10^9$ /l	<2	43	5.0	2.25	0.35
	>2	37	4.6	2.04	

Table (3.9) Comparison between pregnant women according to numbers of children (platelet count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Plt $\times 10^9$ /l	<2	43	213.3	69.13	0.85
	>2	37	210.6	59.58	
PDW	<2	43	14.7	2.54	0.40
	>2	37	15.1	1.72	
MPV	<2	43	9.5	1.61	0.98
	>2	37	9.5	1.62	

Table (3.10) Comparison between pregnant women according to their age group (Hb, Hct, RBCs count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Hb g/dl	<30	47	12.0	1.10	0.07
	>30	33	11.5	1.28	
Hct %	<30	47	35.8	2.87	0.02
	>30	33	34.3	2.98	
TRBCs $\times 10^{12}$ /l	<30	47	4.1	0.36	0.86
	>30	33	4.2	0.35	
MCV fl	<30	47	85.2	4.64	0.03
	>30	33	82.5	6.53	
MCH pg	<30	47	28.5	2.11	0.03
	>30	33	26.8	5.10	
MCHC %	<30	47	33.4	1.53	0.95
	>30	33	33.4	1.42	
RDWSD	<30	47	44.8	4.15	0.18
	>30	33	43.6	3.46	

Table (3.11) Comparison between pregnant women according to their age group (TWBCs count, differential and absolute)

Test	Sample	No	Mean	Std. deviation	P value
TWBCs $\times 10^9$ /l	<30	47	7.3	2.37	0.66
	>30	33	7.6	2.58	
Lymph %	<30	47	27.2	10.86	0.52
	>30	33	25.7	8.42	
Neutr %	<30	47	63.4	11.15	0.94
	>30	33	63.5	11.70	
Lymph $\times 10^9$ /l	<30	47	1.9	0.68	0.67
	>30	33	1.8	0.66	
Neutr $\times 10^9$ /l	<30	47	4.6	2.09	0.47
	>30	33	5.0	2.25	

Table (3.12) Comparison between pregnant women according to their age group (platelet count and indices)

Test	Sample	No	Mean	Std. deviation	P value
Plt $\times 10^9$ /l	<30	47	207.6	64.98	0.47
	>30	33	218.3	64.27	
PDW	<30	47	14.6	2.47	0.09
	>30	33	15.4	1.65	
MPV	<30	47	9.6	1.58	0.84
	>30	33	9.5	1.67	

Chapter Four

Discussion, Conclusion, and Recommendations

4.1. Discussion

The results of this study showed that there was significant decreased in means of Hct, TRBCs, RDWSD, platelets count and lymphocytes percentage and absolute count ($p \leq 0.01$) in pregnant women when compared with control. There was significant increased in means of MCHC, TWBCs and neutrophils percentage and absolute ($p=0.00$) of pregnant women more than control. There was insignificant decreased in means of Hb, MCV, RDWCV and PDW ($p \geq 0.33$) in pregnant women when compared with control, MCH and MPV ($p \geq 0.48$) insignificantly increased in pregnant women when compared to control (tables 3.1, 3.2, 3.3).

There was significant increased in means of MPV ($p=0.01$) according to history of abortion. There was no significant difference in means of Hb, Hct, TRBCs count and indices and, differential and absolute TWBCs count ($p > 0.21$) between with and without abortion(tables 3.4, 3.5, 3.6).

There was significant increased in means of Hct, MCV, and RDWSD ($p < 0.01$) in < 2 more than > 2 children. There was no significant difference in means of Hb, TRBCs, MCH, MCHC, RDWCV, TWBCs count, differential and absolute and platelet count and indices($p > 0.10$) between < 2 and >2 children (tables 3.7, 3.8 and 3.9).

-There was significant increased in means of Hct, MCV, and MCH ($p \leq 0.03$) in < 30 more than > 30 years. There was no significant difference in means of Hb, TRBCs, MCHC, RDWSD, RDWCV, TWBCs count, differential and absolute, and platelet count and indices ($p \geq 0.07$) between < 30 and > 30 years(tables 3.10, 3.11, 3.12).

In previous study in West Bengal, India of hematological parameters in pregnancy the results showed that study group exhibited statistically significant lower values of hemoglobin, PCV of pregnant women compared with the control ($p< 0.05$). While WBC were significantly elevated. The value of neutrophils is higher in the studied group than the control group, but there is no statistical difference between the value of neutrophils in both the study and control groups. In Indian study lymphocytes were lower (Das *et al*., 2013). These results agreed with the results of the current study, significant increased in means of Neutrophils (%) and WBC, significant decreased in means of Hb, PCV and Lymphocytes (%), ($p < 0.05$) in pregnant women when compare with control. The haemodilutory effect commences in the first trimester. The lower limit of the hemoglobin level in normal pregnant women is about 10.5 g/dl, and is thus less than in the non-pregnant state (Firkin *et al*., 1989).

In other previous study of hematological profile of normal pregnant women in Lagos, Nigeria, (Akinbami *et al*., 2013) were reported that hematocrit level was 30.16 % ± 5.55 % , hemoglobin concentration 10.94 ± 1.86 g/dl; white blood cells, 7.81 ± 2.34 $\times 10^9$/L; platelets, 228**.**29 ±65.6$\times 10^9$/L; cell volume 78.30 ±5.70 fl, corpuscular hemoglobin, 28.57 ±2.48 pg; and corpuscular hemoglobin concentration, 36.45 ±1.10 g/dl. When grouped by trimester, the mean ±SD values at third trimester packed cell volume 33.04 % ±3.88%, hemoglobin concentration values were 10.38± 1.72 g/dl, white blood cell concentration 8.37 ±2.15 $\times 10^9$/L, and platelet values were 200.82 ±94.42 $\times 10^9$/L. A statistically significant relationship was found to exist between packed cell volume and white blood cell count with increase in gestational age ($P = 0.010$ and 0.001, respectively) (Akinbami *et al*., 2013). These results agreed with the results of the current study, significant increased in means of Neutrophils

(%) and WBC, significant decreased in means of Hb, P.C.V and Lymphocytes (%), ($p \leq 0.05$) in case when compare with control. Thrombocytopenia in pregnancy represents a particular clinical problem. The different types and causes. Some particular disorders are unique to pregnancy, such as gestational thrombocytopenia of uncertain pathogenesis found in10% of pregnancies, idiopathic thrombocytopenic purpura has the same incidence in pregnancy, pre-eclampsia thrombocytopenia its mechanism is unknown, the HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), thrombotic thrombocytopenic purpura typically occurs before the 24th week and hemolytic uremic syndrome typically occurs within 48 h of delivery. Normal pregnancy is accompanied by a mild neutrophilia; the left shift is mild (only band forms) usually becoming obvious around the middle of pregnancy (Beck, 2009).

In other previous study, assessment of complete blood count of Sudanese pregnant women in Port Sudan city by (Khalil R.K.A., 2012) master thesis Sudan University of Science and Technology, the results indicated that WBC 8.0×10^3/µl±2.1of pregnant women with number of pregnancy between (1-3)pregnancies increased insignificantly (p.value> 0.05) compared to those of pregnancy between(4-7) and (7-10).WBCs 8.0×10^3/µl±2.1 of pregnant women at third trimester increased insignificantly (p.value 0.08)while lymphocytes(mean=25%±7.0) decreased significantly (p.value0.01) than those women at first and second trimesters Neutrophils 66.2%± 8.0 of women at third trimester increased significantly compared to those in first and second trimesters (p.value 0.02),when compared of different trimesters with control the result was WBCs and neutrophils increased significantly at third trimester (p- value 0.00) but for lymphocytes was decreased significantly at third trimester (p- value 0,00)MPV 10.1 fl±1.0 of pregnant women with history

of abortion significantly increased compared to those with no history of abortion (p- value 0.03).RBCs 4.2×10^{6}/µl± 0.5) and HCT 34.5%±3.6) of pregnant women increased significantly (p.value0.01) with regularly visits to clinics (Khalil RKA, 2012). These results completely agreed with the current study. Pregnancy is commonly associated with thrombocytopenia; usually appearing during the third trimester due to poor nutritional intake may result in decreased intake of vitamin B12 and folate resulting in megaloblastic anemia and thrombocytopenia due to decreased platelet production (O'Shaughnessy *et al.*, 2005). In pregnancy increased iron is needed for an increased maternal red cell mass of approximately35%, transfer of 300 mg of iron to the fetus and because of blood loss at delivery. Although iron absorption is also increased, iron therapy is often needed if the hemoglobin (Hb) falls below 10 g/dl or the mean cell volume (MCV) is below 82 fl in the third trimester (Hoffbrand *et al.*, 2006)**.**

4.2. Conclusions

This study was concluded that: there was significant decreased (p≤ 0.01)in means of Hct, TRBCs, RDWSD, lymphocyte percentage and absolute count and platelet count in pregnant women when compared with control**,** significant increased (p=0.00) in means of MCHC, TWBCs and neutrophils percentage and absolute of pregnant women more than control, insignificant decreased (p≥0.33) in means of Hb, MCV, RDWCV, and PDW in pregnant women when compared with control, insignificant increased(p≥0.48) in means of MCH and MPV of pregnant women more than control. There was no significant difference in means of Hb, Hct, TRBCs, and RBCs indices, TWBCs count and differential (p≥0.21), and there was significant increased in means of MPV (p=0.01) of with abortion when compared to without abortion. According to numbers of children there was significant increased (p=0.01) in means of Hct, MCV, and RDWSD in < 2 more than > 2 children, and there was no significant difference (p≥ 0.10) in means of Hb, TRBCs, MCH, MCHC, RDWCV,TWBCs count and differential, and platelet count and indices . According to their age group there was significant increased (p=≤ 0.03) in means of Hct, MCV, and MCH in < 30 more than > 30 years, and there was no significant difference (p≥ 0.18) in means of Hb, TRBCs, MCHC, RDWSD, RDWCV, platelet count and indices, and TWBCs count and differential.

4.3. Recommendations

Follow up of general healthy of pregnant women, CBC should be done regularly during pregnancy, and iron profile should be done when Hb, MCV, and MCHC were less than normal. Normal values should be done in Sudan, to established data base for pregnant women.

References

Akinbami AA, Ajibola SO, Rabiu KA, Adewunmi AA, Dosunmu AO, Adediran A, Osunkalu VO, Osikomaiya BI and Ismail KA. (2013). Hematological profile of normal pregnant women.International journal of women's health ***5***: 227-232.

Bain B.J. (2004). A Beginner's Guide to Blood Cells. 2end edition. London. Blackwell Publishing Ltd.

Beck N. (2009). Diagnostic Hematology. 1st edition. Springer-Verlag London Limited.

Ciesla B. (2007). Hematology in Practice. Philadelphia. Copyright © Davis Company.

Dacie J.V. and Lewis M. (2011). Practical Hematology. 11th edition. London. Elsevier Limited.

Das S, Char D, Sarkar S, Saha TK, Biswas S. (2013). Study of

ematological parameters in pregnancy**.** IOSR-JDMS ***12:*** 1**.**

Firkin F., Chesterman C., Penington D., and Rush B., (1989). De Gruchy's Clinical Hematology in Medical Practice. 5th edition. North America. Blackwell Science Ltd.

Hoffbrand A.V., Katovsky D., and Tuddenham E.G.D. (2005). Postgraduate Hematology. 5th edition. London. Blackwell Publishing.

Hoffbrand A.V., Moss P.A.H. and Pettit J.E. (2006). Essential Hematology. 5th edition. London. Blackwell Publishing.

Khalil R.K.A. (2012). Assessment of complete blood count of pregnant women in Port Sudan city. http://repository. sustech. Edu / handle/ 123456789/1398.

O'Shaughnessy D., Makris M., Lillicrap D. (2005) Practical Hemostasis and Thromposis. 1st edition.USA. Blackwell Publishing Ltd.

Turgeon M. L. (2010). Clinical Hematology Theory and Procedures. 5th edition. USA. Lippincott Williams and Wilkins, a Wolters Kluwer business.

Williams and Wilkins. (2006). Stedman's Medical Dictionary. 2end edition.

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire to measure CBC of pregnant women in Third trimester attended in Khartoum locality

NO ( )

Personal data Name……..

Age……

Occupation…….……

Husband occupation……

Residence……

Month of pregnancy……

NO of pregnancy…….

Abortion: yes ( ) how many times ( ) No ( )

Supplementation intake yes ( ) No ( ) Regular ( ) Irregular ( )

Visit to clinic yes ( ) No ( )

Suffer from disease: Malaria ( ) Anemia ( ) Typhoid ( )

Other……

Previous blood transfusion: yes ( ) When ( ) No ( )

Result: WBC….....RBC…….HGB……..……..

HCT……....MCV……...MCH……..……

MCHC……..…PLT…….LYM%.....

NEUT%.....MXD%.....LYM#.....

NEUT#.....MXD……...RDW…..…..……

PDW…….....MPV……..…….

Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم ومبحث المناعة الدموية

براءة اخلاقية

الإسم :.....

سوف يتم أخذ عينة من الدم (3 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينة بواسطة مطهر.

كل الأدوات المستخدمة لأخذ العينة معقمة و متبع فيها وسائل السلامة المعملية

أوافق أنا المذكور اعلاه أخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ:.....

Appendix (3)

Figure (2.1) Sysmex KX -21N