بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Determination of Complete Blood Cell Count of Sudanese Pregnant Women at Second Trimester- Omdurman locality

تحديد صورة الدم الكاملة عند النساء الحوامل فى مستشفيات محلية امدرمان خلال الفترة الثانية للحمل

A dissertation Submitted in partial Fulfillment for requirements of M.Sc Degree in Medical Laboratory Science (Hematology and Immunohematology)

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بسم الله الرحمن الرحيم

قال تعالي:

﴿وَيُحِلُّ لَهُمُ الطَّيِّبَاتِ وَيُحَرِّمُ عَلَيْهِمُ الْخَبَائِث﴾

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Dedication

To..

My life beats, for whom I work to make their dreams, my parents **Mona ,** and my father **Salah**…

To ..

The soul of my favorite uncle **Aydroos ,** and for who have the biggest credit for complete this study ..**Abd Elkhalig Naser.**

To..

My biggest lover …who helped me very mutch in this study my husband **Mohamed ..**

To..

My life brightness, without whom I could not continue smile, my brothers **Amged , Ashraf،** my lovely sister **Hadeel** and for my life **friends** , they are filling all my days ..**Tabo ,hota and M.Hamza.**

To ..

The absolutely necessary person..the gentle **reader** ..

Hoyam

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Allah enabled me to conduct this study by his blessing therefore thanks for my god **Allah** firstly and lastly..

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Last thanks for **everyone** helped me in my research ..

HOYAM

Abstract

This is a case control study , conducted in Omdurman Locality during the period from February to May 2015 , to determine CBC (Hb, RBCs, HCT , MCV , MCH ,MCHC, Plets , WBCs, leukocyte differential count , RDW and MPV) of (80) healthy Sudanese pregnant women at second trimester as case and (40) non pregnant women at matched age in Omdurman locality were used as control in this study . Pregnant women at second trimester were informed about the study and agreed for participatin as cases . A questionnaire was designed to collect information about the study group such as demographic data ,age , number of pregnancies , month of pregnancy ,history of abortion and whether they visit the clinic regularly .

2.5 ml venous blood was collected in EDTA anticoagulant container . automated hematological analyzer (Sysmex KX-21N) was used to measure CBC and the result were analyzed by independent T test of SPSS computer program .

This study was concluded that : There was significant decrease in Hb, HCT, RBCs count, lymph % and absolute lymph and PDW in pregnant women when compared with control group ($p.\leq 0.02$) .and significant increase ($p.=0.00$) in, TWBCs neut% and absolute neut count in pregnant women when compared with control group. There was no significant difference in MCH, MCHC, Plets count , MCV, RDWcv, and MPV in pregnant women when compared with control group. There was significant decrease in Hb, HCT, RBCs count, MCH ($p.\leq 0.02$) and significant increase in TWBCs, Neut% , absolute Neut and MPV ($p.=0.00$). No significant difference in MCV, MCHC, Lymph % , absolute lymph ,plets count ,PDW and RDW in abortion pregnant women when compared with non abortion pregnant women($p.\geq 0.08$). There was significant decrease in Hb and absolute Neut ($p.\leq 0.04$)and significant increase in lymph

% and PDW (p. ≤ 0.03) .also there was no significant difference in HCT, MCV, MCH, MCHC, RDWcv, TWBCs, Neut%, Plets count, MPV,RBCs count in pregnant women >30 years in age when compared with pregnant women <30 years in age(p.≥ 0.06). There was no significant difference in hematological parameter between pregnant women with less than 3 pregnancies and pregnant women with more than 3 pregnancies **(**p. ≥ 0.10) (the mean of pregnancy 1-6 pregnancies) .

المستخلص

اجريت هذه الدراسه بطريقة الحالات الافراديه المقترنه بحالات ضابطه فى محليه امدرمان فى الفترة الثانيه من الحمل . تم اختيار ثمانين من النساء الحوامل وفقا لنظام الاختيار المحدد وعوملن كعينات اختياريه واربعين من النساء غير الحوامل فى نفس الفئه العمريه وعوملن كعينات ضابطه بعد اخذ 2.5 ملى لتر عينه دم ورديه من كل متبرع ووضعت فى اناء بلاستيكى يحتوى على مانع التجلط (ادتا) وتم اختبارها لقياس صورة الدم الكامله بستخدام جهاز تحليل الدم الالى .ثم تم تحليل النتائج بستخدام الفرق بين المتوسطين غير المعتدين فى برنامج الحزم الاحصائيه للعلوم الاجتماعيه المحوسب .

إستنتج من هذه الدراسه ان هناك نقص دات دلاله معنويه فى نسبة خضاب الدم ،الدم المكدس ،عدد كرات الدم الحمراء ،ألنسبه المئوية والعدد المطلق للخلايا اللمفاويه وانتشار الصفائح الدمويه عند النساء الحوامل مغارنة مع النساء غير الحوامل .وزياده معنويه فى عدد كرات الدم البيضاء والنسبه المئويه والعدد المطلق للخلايا المعده عند النساء الحوامل مغارنة بالنساء غير الحوامل . كما توجد زياده معنويه فى عدد كرات الدم البيضاء والنسبه المئويه والعدد المطلق للخلايا المعده ومتوسسط حجم صفيحة الدم الواحده عند النساء اللاتى تعرضن لاسقاط مغارنة باللاتى لم يتعرضن لاسقاط . كما يوجد نقص ذات دلاله معنويه فى نسبة خضاب الدم وعدد كرات الدم الحمراء ، ومتوسط خضاب الدم فى الخليه الواحده عند النساء اللاتى تعرضن لاسقاط مغارنة باللاتى لم يتعرضن لاسقاط . بناءا على عمر المراة الحامل فيوجد نقص ذات دلاله معنويه فى نسبة خضاب الدم والعدد المطلق للخلايا المعده وزيادة معنويه فى النسبه المئويه للخلايا اللمفاويه وانتشار الصفائح الدمويه عند النساء اكبر من 30 سنه مغارنة بالنساء الاصغر من 30 سنه. كما لايوجد تغير ذا دلاله معنويه فى الصوره الكامله للدم عند النساء اللتى حملن اقل من 3 مرات مغارنة بالنساء اللاتى حملن اكثر من 3 مرات (متوسط مرات الحمل من مره الى 6 مرات).

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List of Abbreviations

CBC	Complete Blood Count
RBCs	Red Blood Cell
Hb	Hemoglobin concentration
HCT	Hematocrit
PLTs	Platelet
MCV	Mean Cell Volume
MCH	Mean Cell Hemogolobin
MCHC	Mean Cell Hemogolobin Concentration
NEUT	Neutrophils
LYM	Lymphocyte
MIX	Mixed monocyte ,esinophil and basophil
IL	Interleukin
MPV	Mean Platelet Volume
RDWcv	Red Distribution Width by Coefficient of Variation
PDW	Platelet Distribution Width
LCD	Liquid Crystal Displayer
EDTA	Ethylene Diamine Tetra Acetic Acid
TPO	Thrombopoietin
PMN	Polymorph Nuclear

CFU-MEGA	Colony Forming Unit –megacarocyte
CFU-GM	Colony Forming Unit-Ganrulocyte,Monocyte
CFU-G	Colony Forming Unit-Garnulocyte
CFU-E	Colony Forming Unit-Erythrocyte
G-CSF	Ganrulocyte Colony Stimulating Factor
GM-CSF	Ganrulocyte, Monocyte Colony Stimulating Factor
BFU-E	Burst Forming Unit-Erythrocyte
IgE	Immunoglobulin E
RNA	Ribonucleic acid
DNA	Deoxy Ribonucleic acid
NADPH Hydrogenase	Nicotinamide Adenine Dinucleotide Phosphate

Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

Blood is bodily fluid in animals that delivers necessary substance such as nutrient and oxygen to the cells and transports metabolic waste products away from those same cells . Anemia is serious public health problem having a direct bearing on physical development. anemia is present when Hb level in blood is bellow the lower extreme of normal range of the age and sex of individual .A common error leading to misdiagnosis of anemia is failure to refer to normal range (firkin *et al*.,1989).There are many causes of anemia ,blood loss(main causes) impaired red cell production ,inadequate supply of nutrition essential for erythropoieses ,anemia associated with chronic disorder renal diseases ,increase RBC destruction and due to replacement of normal bone marrow by leukemia (Hoffbrand *etal,1993).*Diagnosis of anemia; by measuring of complete blood count (CBC) thin blood film ,biochemical test ,especial test ,anemia can be classify in to tow categories morphological classification which include (hypo chromic microcytic anemia ,norm chromic normocytic anemia ,microcytic anemia) on basis of MCV,MCH,MCHC .and etioloyical classification. Normal pregnancy is characterized by profound changes in nearly every organ system to accommodate the demands of the feto-placental unit. The most significant hematological changes are physiologic anemia, neutrophilia, mild thrombocytopenia. The pregnancy-associated changes in plasma volume, red blood cells, white blood cells and platelets(Reinhold *et al.,*2007).

Condition of pregnancy characterized by reduction in the concentration of hemoglobin in the blood .it may be physiologic or pathologic; in physiologic anemia of pregnancy the reduction in concentration result from dilution because the plasma volume expands more than the erythrocyte volume .the hematocrit in

pregnancy normally drops several point blow it is pregnancy level . In pathologic anemia of pregnancy the oxygen-carrying capacity of the blood is deficient because of disordered erythrocyte 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 from iron ,folic acid, or vitamin B_{12} or from sickle cell or another chronic disease, malignancy ,chronic malnutrition or exposure to toxin. Destruction of erythrocytes may result from inflammation disease , micro angiopathy or hematologic disease in which the erythrocytes are abnormal . Excessive loss of erythrocyte through bleeding may result from abortion , bleeding ,hemorrhoid ,intestinal parasite such as hook worm ,placenta previa and abruption placenta or post partum uterine atone (Williams and Wilkins,2006).

1.2 Hemopoieses and Sites of Hematopoiesis :-

The formation of blood cells (hemopoiesis) is determined by the interaction of multiple genes and involves cytokines and other protein factors (Reinhold *et al.,*2007).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. During infancy and childhood, the marrow of all bones contributes to hematopoiesis. 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 hemolytic anemia's and in some myelo-proliferative disorders), the fatty marrow as well as the spleen and liver can resume the

production of blood cells. This situation is called extra modularly hematopoiesis (Reinhold *et al.,*2007).

1.2.1 Stromal Cells:-

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 (Reinhold *et al.,*2007).

1.2.2 Hematopoietic Stem Cells:-

All hematopoietic cells of the organism derive from pluripotent stem cells that are capable of both self-renewal and differentiation into all hematopoietic lineages. one stem cell provides progenitor cells for myelo- and monopoiesis, erythropoiesis, megakaryopoiesis, and lymphopoiesis (Reinhold *et al.,*2007).

1.2.3 Erythropoiesis :- 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 cell. Erythropoiesis begins at the level of the multipotent stem cell which

then undergoes commitment and differentiation. Listed as follows are the stages of erythroid differentiation:

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

Remnants of ribosomal RNA can be visualized in reticulocytes; no nucleus is present in the mature red cell. (Reinhold *et al.,*2007).

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 hemoglobin levels in the blood and tissue hypoxia, the production of erythropoietin by the kidneys is increased. When the serum levels of erythropoietin are increased, both the rate and the speed of erythropoiesis increase. In states of chronic tissue hypoxia (e.g., in hemolytic anemia's) the proportion of the marrow devoted to erythropoiesis expands and sometimes transforms a large portion of the fatty marrow into active hematopoietic marrow (Reinhold *et al.,*2007).

1.2.3.1 Erythropoietin:-

Erythropoiesis is regulated by the hormone erythropoietin. The erythropoietin gene contains a hypoxiaresponse erythropoietin gene contains a hypoxiaresponse element at its 3' end. Erythropoietin is a heavily glycosylated polypeptide of 165 amino acids with a molecular weight of 34 kDa. Normally,

90% of the hormone is produced in the peritubular element at its 3' end. Erythropoietin is a heavily glycosylated polypeptide of 165 amino acids with a molecular weight of 34 kDa. Normally, 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere. There are no preformed stores and the stimulus to erythropoietin production is the oxygen (02) tension in the tissues of the kidney Erythropoietin production therefore increases in anemia , when haemoglobin for some metabolic or structural reason is tamable to give up 02 normally, when atmospheric 02 is low or when defective cardiac or pulmonary function or damage to the renal circulation affects 02 delivery to the kidney. Erythropoietin stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoiesis (Hoffband *et al.*,2006).

1.2.4 The red blood cell

In order to carry haemoglobin into close contact with the tissues and for successful gaseous exchange, the red cell, 8 μm in diameter, must be able: to pass repeatedly through the microcirculation whose minimum diameter is 3.5 μm, to maintain haemoglobin in a reduced (ferrous) state and to maintain osmotic equilibrium despite the high concentration of protein (haemoglobin) in the cell .(Hoffband *etal*,2006).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 hemolytic anemia. The outer layer is covered with mucopolysaccharides that form part of the structure of blood group antigens. The *N*-acetylneuraminic acid found in these glycoproteins results in a negative charge of the cell surface. 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 d. The necessary energy for red cell

metabolism is supplied by the Embden-Meyerhof pathway, which generates adenosine triphosphate by metabolizing glucose to lactate. This anerobic process also results in the formation of nicotinamide-adenine dinucleotide, which is essential for the reduction of methemoglobin to functionnally active hemoglobin which 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 feces. Part of these hemoglobin degradation products are reabsorbed and excreted via urine as urobilin and urobiliogen. (Reinhold *et a*l.,2007).

1.2.5 Hemoglobin:-

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

- Hemoglobin A : major adult hemoglobin (96–98%).
- Hemoglobin F : predominant during fetal development, 60–80% at birth, 0.5–0.8% during adult life.
- Hemoglobin A2: normally 1.5–3%.

The hemoglobin molecule has a molecular weight of 64,500 and consists of four polypeptide chains, each carrying a heme group. Iron(Fe^{2+}) is supplied from serum transferrin and combines with protoporphyrin to form heme. One heme molecule then binds with one globin chain to form the hemoglobin molecule that avidly binds oxygen (Reinhold *et al.*,2007).

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

- The blood flow, which is controlled by the heart beat volume and the constriction or dilatation of peripheral vessels.
- The oxygen transport capacity, which depends on the number of red blood cells and the hemoglobin concentration.
- The oxygen affinity of hemoglobin. (Reinhold *et al.*,2007).

In anemic 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.(Reinhold *et al.*,2007).

1.2.6 Neutrophil (polymorph):-

This cell has a characteristic dense nucleus consisting of between two and five lobes, and a pale cytoplasm with an irregular outline containing many fine pink-blue (azurophilic) or grey-blue granules The granules are divided into primary, which appear at the promyelocyte stage, and secondary(specific) which appear at the myelocyte stage and predominate in the mature neutrophiL Both types of granule are lysosomal in origin: the primary contains myeloperoxidase, acid phosphatase and other acid hydrolases; the secondary contains collagenase, lactoferrin and lysozyme The lifespan of neutrophils in the blood is only 6-10h (Hoffband *et al.*,2006).

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, aminopeptidase, 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 (Reinhold *et al.*,2007).

1.2.7 Monocytes:-

These are usually larger than other peripheral blood leucocytes and possess a large central oval or indented nucleus with clumped chromatin. The abundant cytoplasm stains blue and contains many fine vacuoles, giving a ground-glass appearance. Cytoplasmic granules are also often present. The monocyte precursors in the marrow (monoblasts and promonocytes) are difficult to distinguish from myeloblasts and monocytes (Hoffband *et al.*,2006).

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) (Reinhold *et al.*,2007).

1.2.8 Eosinophils:-

These cells are similar to neutrophils, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclear lobes . Eosinophil myelocytes can be recognized but earlier stages are from indistinguishable neutrophil precursors. They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation (Hoffband *et al.*,2006).

Eosinophils, which make up 1–4% of the peripheral blood leukocytes, In absolute terms, eosinophils number up to 400/μL. Eosinophilic cells can first be recognized at the myelocyte stage. Eosinophils have a role in the defense against certain tumors (Reinhold *et al.*,2007).

1.2.9 Basophils:-

Basophils are seen less frequently than eosinophils; under normal conditions, fewer than 100 cells/μL are found in the peripheral blood (Reinhold *et al.*,2007). These are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granuleswhich overlie the nucleus and contain heparin and histamine. In the tissues they become mast cells. They have immunoglobulin E (IgE) attachment sites and their degranulation is associated with histamine release (Hoffband *et al.*,2006).

1.2.10 Megakaryopoiesis:-

Platelets are small cell fragments (average size 3–4 μm) that are important for hemostasis and coagulation. The normal platelet count is between 150,000 and450,000/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 megakaryocyte 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 megakaryocyte progenitor (CFU-Mega), 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 occur under the influence of thrombopoietin (TPO). 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.) (Reinhold *et al.*,2007).

1.2.11 Pregnancy and Hematologic Changes in Pregnancy:-

Normal pregnancy is characterized by profound changes in nearly every organ system to accommodate the demands of the feto-placental unit. The most significant hematological changes are physiologic anemia, neutrophilia, mild thrombocytopenia. the pregnancy-associated changes in plasma volume, red blood cells, white blood cells and platelets (Reinhold *et al.,*2007).

1.2.11.1 Pregnancy trimesters:-

1.2.11.1.1 Pregnancy in first trimesters:-

At the end of the first month the embro is about a third of an inch long and it is head and trunk plus the beginning of arms and legs .have started to develop .the embryo receive nutrients and eliminates waste through the umbilical cord and placenta ,By the end of the first month ,the liver and digestive system begin to deveop ,and the heart start to beat ,second month in this month the heart start to

pump and the nervous system begins to develop in (2.5cm) long fetus has acomplete cartilage skeleton ,which is replace by bone cells by month end .Arms, leges,and all of the major organs begin to appear.

Facial features begin to form third month by now, the fetus has grown to (10cm) and weigh alittle more than an ounce (28g). now the major blood vessels and the roof of the face start to take on amore recognizable human appearance ,finger and toes appear, All the major organ are now beginning to form ,the kidney are now functional and the four chamber 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 10.5g /dl and is thus less than in the non-pregnant state (Firkin *et al.*, 1989).

1.2.11.1.2 Pregnancy in Second Trimester:-

Fourth month the fetus begins to kick and swallow .although most women still cannot feel the baby move at this point .Now the fetus can hear and urinate .and has established sleep .wake eyeless all organs are now fully formed although they will continue to grow for the next five month, the fetus has skin, eye brows, and hair . Fifth month now weighing up to (454g) and measuring in 20-30cm 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 heart beat with a stethoscope sixth move even though it is lungs are not fully developed a fetus born during this month can survive with intensive care weighing (454-681 g),the fetus is red ,wrinkly and covered with fine hair all over it is body .the fetus will grow very fast during this month as it is organs continue to develop (Williams and Willkin,2006).

1.2.11.1.3 Pregnancy in 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 (1-3 kg) by now. Now the fetus can look around it is watery womb with open eyes. Eighth month growth continues but slow down as the baby begins to take up most of the room inside the uterus. Now weighing (1.8-2.3kg) and measuring (40-45 cm) long ,the fetus may at this time prepare for delivery next month by moving in to the head down position .

Ninth month adding (229 g) a week as the due to date approaches the fetus drops lower in to the mother's abdomen and prepares for onset of lobar ,which may begin any time between the 37^{th} and 42^{nd} week of gestation . Most healthy babies will weigh (2.7-4 kg) at birth (Williams and Wilkins,2006).

1.2.11.2 The Hematologic Changes in Pregnancy:-

1.2.11.2.1 Plasma Volume:-

Plasma volume increases by 10–15% at 6–12 weeks of gestation (Lund and Donovar,1967) . expands rapidly until 30–34 weeks, after which there is only a modest rise. The total gain of plasma volume at term averages 1100–1600 mL and results in a plasma volume of 4700–5200 mL, 30–50% above that found in non pregnant women.(Pritchard ,1965).

Plasma volume decreases immediately postpartum, then increases again 2–5 days after delivery, possibly because of a simultaneous rise in aldosterone secretion. Plasma volume then decreases; it is still elevated by 10–15% above non pregnant levels at3 weeks postpartum, but is usually at normal non pregnant levels at6 weeks postpartum. During pregnancy, plasma rennin activity is typically increased and aerial natriuretic peptide levels are slightly reduced, suggesting that the increase in plasma volume represents under filling due to systemic

vasodilatation and the ensuing rise in vascular capacitance, rather than true blood volume expansion, which would produce the opposite hormonal profile (low plasma rennin activity, elevated aerial natriuretic peptide) (Pritchard ,1965).

Furthermore, the degree of sodium retention is physiologically regulated, as increasing sodium intake does not produce further volume expansion (Lindheimer and Katz,1973*).*

1.2.11.2.2 Red Blood Cells:-

Red blood cell mass begins to increase at 8–10 weeks of gestation and steadily rises by 20–30% (250–450 mL) above non pregnant levels by the end of pregnancy

in women receiving iron supplementation (Metcalfe *et al.*,1988*).* Among women not on iron supplements, the red cell mass may only increase by 15–20%. Erythrocyte life span is slightly decreased during normal pregnancy. Erythropoietin levels increase by 50% in normal pregnancies and vary according to the presence of pregnancy complications (Harstad *et al* .,1992).The increased plasma erythropoietin induces the rise in red cell mass, which partially supports the higher metabolic requirement for oxygen during pregnancy (Milman *etal* ,1997) Mean corpuscular volume decreases during pregnancy and averages 80–84 fL in the third trimester (Whittaker *et al.*,1996).

1.2.11.2.3 Platelet Count:-

Although platelet counts remain in the normal non pregnant range in most women during uncomplicated pregnancies (Giles and inglis,1981*).*mean platelet counts of pregnant women may be slightly lower than in healthy non pregnant women (Matthews *et al.*,1990)Serial platelet counts during uncomplicated pregnancies may or may not decrease (Ahmed *et al.*,1993).but the mean values in these groups do not necessarily reflect both increases and decreases in individual women (Minakami *et al.*,1996*).* The lower limit of normal platelet counts in

pregnancy has been reported to be 106,000–120,000 platelets/μL. The most significant obstetrical consideration concerning platelet physiology in pregnancy is thrombocytopenia, which may be related to complications of pregnancy (e.g., severe preeclampsia), medical disorders (e.g., idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura-hemolytic uremic syndrome), or gestational. Gestational or incidental thrombocytopenia is characterized by mild asymptomatic thrombocytopenia occurring in the third trimester in a patient without any history of thrombocytopenia (other than in a prior pregnancy). Platelet counts are typically greater than 70,000/μL (George *et al.*,1996*).*

1.2.11.2.4 White Blood Cells:-

Pregnancy is associated with leukocytosis, primarily related to increased circulation of neutrophils. The neutrophil count begins to increase in the second month of pregnancy and plateaus in the second or third trimester, at which time the total white blood cell counts ranges from 9000 to15,000 cells/μL(Kuvin and Brecher,1962*)* Data from two series reported mean white blood cell counts of 10,000–16,000 cells/μL in laboring patients, with an upper level as high as 29,000 cells/μL (Molberg *et al* .,1994).

The white blood cell count falls to the normal nonpregnant range by the sixth day postpartum. Dohle bodies (blue staining cytoplasmic inclusions in granulocytes) are a normal finding in pregnant women. In healthy women with normal pregnancies, there is no change in the absolute lymphocyte count and no significant changes in the relative numbers of T and B lymphocytes, the monocyte count is generally stable; the basophil count may slightly decrease and the eosinophil count may slightly increase. Normal pregnant women can have a small

number of myelocytes or metamyelocytes in the peripheral circulation (Kuhnert *et al.*,1998).

1.2.11.3 Anemia in pregnancy:-

Determining a good definition of anemia in pregnant women is not Straight forward, given the pregnancy-associated changes in plasma volume and red cell mass, normal differences in hemoglobin concentrations between women and men, ethnic variation between white and black women, and the frequent use of iron supplementation in pregnancy. The Centers for Disease Control and Prevention has defined anemia as hemoglobin levels of less than 11 g/dL (hematocrit less than 33%) in the first and third trimesters and less than 10.5 g/dL (hematocrit less than32%) in the second trimester .Since hemoglobin and hematocrit levels are lower in African-American adults, the Institute of Medicine recommends lowering of the hemoglobin cutoff level by 0.8 g/dL in this population .Women with hemoglobin values below these levels can be considered anemic and should undergo a standard evaluation. Sixteen to twenty-nine percent of pregnant women become anemic in the third trimester (Bailit *et al.*,2007*).*

Severe anemia with maternal hemoglobin below 6 g/dL has been associated with reduced amniotic fluid volume, fetal cerebral vasodilatation, and no reassuring fetal heart rate patterns .Increased risks of prematurity, spontaneous abortion, low birth weight, growth restriction, and fetal death have also been reported (Carles *et al* .,2003).

1.2.11.3.1 Iron Requirements:-

In a typical singleton gestation, maternal iron requirements average close to 1000 mg over the course of pregnancy: approximately 300 mg for the fetus and placenta and approximately 500 mg, if available, for the expansion of the maternal hemoglobin mass. Two hundred milligrams is shed through the gut, urine, and skin. Since most women

do not have adequate iron stores to handle the demands of pregnancy, iron is commonly prescribed as part of a prenatal multivitamin or as a separate supplement . In general, women taking iron supplements have a mean hemoglobin concentration that is 1 g/dL greater than that of women not taking supplements (Hoffband *et al.*,2006).

1.2.11.3.2 Folate Requirements:-

The increase in red cell mass also necessitates an increased folic acid requirement. In non pregnant women, the daily folic acid requirement is50–100 mg/d. However, because folate deficiency is associated with neural tube defects (and possibly other birth defects) as well as macrocytic anemia, all women of reproductive age are advised to consume 0.4 mg of folic acid daily (Hoffband *et al.*,2006).

the most common type of anemia in pregnancy is iron deficiency anemia

1.2.11.3.3 Iron deficiency anemia

Iron deficiency is still the world's most common nutritional deficiency, and generally, iron deficiency anemia is the most prevalent form of anemia. The major risk groups for iron deficiency include women of childbearing age, pregnant women, and lactating postpartum women (Hoffband *et al.*,2006).

Causes of iron deficiency anemia usually by 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 alitre 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. 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 haemoglobin (Hb) falls

below 10 g/dL or the mean cell volume (MCV) is below 82 fL in the third trimester (Hoffband *et al.*,2006).

1.2.11.3.4 Megaloblastic anemia of pregnancy:-

This is a group of anemia's in which the erythroblasts in the bone marrow show a characteristic abnormality-maturation of the nucleus being delayed relative to that of the cytoplasm.. This is caused by deficiency of vitamin Bl2 or folate. Less commonly, abnormalities of metabolism of these vitamins or other lesions in DNA synthesis may cause an identical hematological appearance (Hoffband etal,2006).

Megaloblastic anemia during pregnancy result from an inadequate intake of folate to meet the increased requirement of pregnancy .A small proportion of cases are due to latent celiac disease first becoming manifest during pregnancy ,rare cases are due to thee 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).*

1.2.11.3.5 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 increase three fold. There is a progressive fall in serum folate values subnormal levels occurring in about 50 percent of patient 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 percent of pregnant women in late pregnancy. In the occasional case, further progression to fronk megaloblastic anemia occurs. Other factor ,bide fetal demand that may contribute to the development of anemia include iron deficiency ,coexistent hemolytic anemia ,urinary tract and other infection ,anticonvulsant and trimethoprim therapy, and altered intestinal absorption of folate (Firkin et al.,1989).

1.2.11.3.6 Prevention of anemia in pregnancy:-

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

1.2.12 Previous Study :-

1.2.12.1 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 Hb ,PCV,of pregnant women compared with the control ($P<0.05$).while WBC were significantly 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 neutrophil in both the study and control groups .in this present study lymphocyte counts were lower in studied group than in control comparison of heamatological indices in pregnant women and control (Das et al., 2013).

1.2.12.2 Previous Study in Nigeria:-

In this study Akinbami and other authors studied of hematological profile of normal pregnant women in lagos ,Nigeria they were found that , 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 0.001respectively). However, there was no statistically significant association between platelet count and increase in gestational age (P=0.296) (Akinbami *et al*., 2013)

1.2.12.3 Previous study in sudan :-

In previous study ,assessment of complete blood count of Sudanese pregnant women in port sudan city by Khalil (2012).the results indicated that WBC (mean = 8.0×10^9 /L$\pm$2.1) of pregnant women with number of pregnancy between (1-3) pregnancies increase insignificantly (p.> 0.05) compared to those of pregnancy between (4-7) and (7-10) WBCs (mean =8.0×10^9 /L$\pm$2.1) of pregnant women at third trimester increased insignificantly (p.0.08) while lymph (mean =25%$\pm$7) decrease significantly (p.0.01) than those women at first and 2nd trimester (p.0.02) when compared of different trimester with control the result was WBCs and Neut increased significantly (p.0.00) but lymph was decrease significantly (p.0.00) MPV (mean =10.1/ fL $\pm$1.0) of pregnant women with history of abortion significantly increased compared to those with no history of abortion (p.0.03) RBC(mean =4.2×10^{12} /L $\pm$0.5) and HCT (mean = 34.5 % $\pm$3.6) of pregnant women increased significantly (p.0.01) with regularly visit to clinic center (Khalil, 2012).

1.3 Rationale:-

Pregnancy is often associated with many hematological complication in the medical conditions in pregnant women.Therefore the study was designed to determine the effect of pregnancy at second trimester on the complete blood cells count and hematological changes occurs in pregnant women at 2^{nd} trimester on Omdurman locality. That might effect in pregnant women during pregnancy and lobular. That to prevent the complication which can be occurs at this time.

1.4 Objectives:-

1.4.1 General objective:-

To measure complete blood cells count of Sudanese pregnant women in the second trimester at Omdurman locality.

1.4.2 Specific objective:-

- To determine RBCs and RBCs indices, Hb, PCV, WBCs and it's differential ,PLTs and PLTs indices in pregnant and non-pregnant women .
- To compare the mean of hematological parameter of pregnant women according to age, number of pregnancy and abortion.
- To compare the PLTs and it's indices between abortion and non abortion pregnant women.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1. Study approach

An automation method was used to measure complete blood cells count in Sudanese pregnant women at second trimester during a period from February to May 2015.

2.2 Study Design

Case –control study design.

2.3 Study Area

This study was carried out in Omdurman locality.

2.4 Study Population

Sudanese pregnant women at second trimester in Omdurman locality.

2.5 Inclusion criteria

Specimens were collected from pregnant women at second trimester,which have not any disease may effect in the reult and all age groups were included.

2.6 Exclusion criteria

Non pregnant women and pregnant women on other trimester were excluded.

2.7 Sample size

120 samples (80 samples from pregnant women, 40 from non pregnant women).

2.8 Method of data collection

Data were collected using structural interviewing questionnaire, which was designed to collect and maintain all information concerning each case examined.

2.9 Collection of Samples

Samples were collected by using dry, plastic syringes, tourniquet was used to make the veins more prominent, blood samples (2.5ml) was collected in EDTA containers from each volunteer under septic condition, for CBC.

2.10 Procedure of CBC:-

Fully automated multichannel instruments require only that an appropriate blood sample is present to the instrument and usually measure from 8-20 component for the basic CBC and WBCs differential impedance counting system depends of the fact that Red cells are poor conductor of electricity , where as certain diluents are good conductors (Dacie and lewis, 2011). The reagent for operation were checked ,then the power switch was turn on auto rise and background check were automatically performed then three level of control (low count, normal and high) were applied after selection whole blood mode of analysis sample number were introduced by pressing sample number keys then enter key was pressed ,after that sample was mixed carefully the tab bring in close contact with sample probe and the start key was pressed, after the required volume of blood were aspirated ,then tube was removed ,result was displayed in the screen and was printout .(Sysmex Americaine ,2003).

2.11 Materials:-

Instrument and equipment:-

1- EDTA container.
2- Disposable syringes.
3- Tourniquet.
4- Cotton wool.
5- Sysmex KX,21N.

2.12 Reagents:-

1. Cell pack (diluents).
2. Stromatolyser .
3. Cell clean .

2.13 Method:-

Complete blood cell count (CBC):- Hb, RBCs, WBCs, MCH, MCV, MCHC, HCT, PLTs and it's indices was tested by automated method (Sysmex).

2.14 Quality control of Sysmex :-

Hematology analyzer provide quick and accurate results in most situation. However , false results related either to platelets or other parameter from complete blood cells count may be observed in several instances , fale low WBCs count may be observed because of observed because of agglutination in the preence of EDTA.

2.15 Ethical Considerations

The study was approved by the Medical Laboratory College Committee –SUST. Written consent was obtained from the participants after they been informed with the objective, benefits and expected outcomes of study .The participants were assured that the collected information will be kept confidential and will not be used for any other purpose other than this study.

2.16 Statistical analysis:-

The effect of Age, history of abortion, and the mean of Hb , WBCs, RBCs, PLTs, HCT in pregnant women was test by using SPSS version14.0.(T-independent test ,mean $\pm$ SD).

Chapter Three

Results

Chapter Three

Results

There was significant decrease in Hb, HCT, RBCs count, lymph % , absolute lymph and PDW in pregnant women when compared with control group .and significant increase in TWBCs, neut% and absolute neut count in pregnant women when compared with control group. there was no significant difference in MCH, MCHC, Plets count , MCV, RDWcv, and MPV in pregnant women when compared with control group.(table 3.1,3.2,3.3).

There was significant decrease in Hb, HCT, RBCs count, MCH and significant increase in TWBCs, Neut% , absolute Neut and MPV. No significant difference in MCV, MCHC, Lymph % , absolute lymph ,plets count ,PDW and RDW in abortion pregnant women when compared with non abortion pregnant women.(table 3.4,3.5,3.6).

There was significant decrease in Hb and absolute Neut and significant increase in lymph % and PDW also there was no significant difference in HCT, MCV, MCH, MCHC, RDWcv, TWBCs, Neut%, Plets count, MPV, RBCs count in pregnant women >30 years in age when compared with pregnant women <30 years in age.(table 3.7,3.8,3.9).

There was no significant difference in hematological parameter between pregnant women with less than 3 pregnancy and pregnant women with more than 3 pregnancies.(table 3.10,3.11,3.12).

Table (3.1) Hb , HCT, RBCs count and RBCs indices in pregnant women compared with non pregnant women.

Parameter	Sample	No	Mean ±Std	p.vaule
Hb g/dl	Pregnant	80	10.7± 1.4	0.00
	Control	40	12.8± 0.9	
HCT %	Pregnant	80	34.4± 4.4	0.00
	Control	40	40.9± 2.8	
RBCs $\times 10^{12}$ /L	Pregnant	80	3.9± 0.5	0.00
	Control	40	4.6± 0.3	
MCV/fl	Pregnant	80	87.5± 8.1	0.32
	Control	40	86.1± 5.3	
MCH /pg	Pregnant	80	27.3± 3.3	0.87
	Control	40	27.4± 2.3	
MCHC /g/dl	Pregnant	80	31.4± 1.9	0.89
	Control	40	31.5± 1.9	
RDWcv	Pregnant	80	13.5± 2.3	0.48
	Control	40	13.3± 0.5	

Significane level at p.value $p<0.05$.

Table (3.2) T.WBCs count, differential and absolute in pregnant women compared with non- pregnant women .

Parameter	Sample	No	Mean ± Std	p.value
T.WBCs ×10^9 /L	Pregnant	80	7.6± 2.3	0.00
	Control	40	4.8± 0.6	
Lymphcytes %	Pregnant	80	29.7± 31.5	0.02
	Control	40	41.3± 2.7	
Neutrophils %	Pregnant	80	66.9± 8.0	0.00
	Control	40	51.7± 4.9	
Absolute lymphocytes	Pregnant	80	1.8± 0.5	0.00
	Control	40	2.9± 0.6	
Absolute neutophils	Pregnant	80	5.2± 1.7	0.00
	Control	40	2.5± 0.5	

Significane level at p.value p<0.05.

Table (3.3) platelets count and it's indices in pregnant women compared with non pregnant women.

Parameter	Sample	No	Mean ± Std	p.value
Platelets ×10 /L	Pregnant	80	230 ± 64.7	0.25
	Control	40	241 ± 39.7	
PDW	Pregnant	80	13.4 ± 1.9	0.00
	Control	40	14.2 ± 1.2	
MPV	Pregnant	80	9.8 ± 0.9	0.10
	Control	40	9.6 ± 0.4	

Significane level at p.value p<0.05.

Table (3.4) Effect of absorption on Hb , HCT, RBCs count and it's indices in pregnant women at second trimester.

Parameter	Sample	No	Mean ± Std	p.value
Hb g/dl	No abortion	86	11.8 ± 1.4	0.00
	Abortion	34	10.3 ± 1.6	
HCT %	No abortion	86	37.6 ± 4.6	0.00
	Abortion	34	34.0 ± 5.2	
RBCs×10^{12}/l	No abortion	86	4.2 ± 0.5	0.00
	Abortion	34	3.8 ± 0.6	
MCV /fl	No abortion	86	87.1 ± 7.3	0.83
	Abortion	34	86.8 ± 7.8	
MCH /pg	No abortion	86	27.6 ± 3.2	0.02
	Abortion	34	26.3 ± 4.1	
MCHC /g/dl	No abortion	86	31.5 ± 1.8	0.39
	Abortion	34	31.2 ± 2.2	
RDWcv	No abortion	86	13.4 ± 1.7	0.30
	Abortion	34	13.8 ± 2.4	

Significane level at p.value $p<0.05$.

Table (3.5) Effect of absorption on T.WBCs count,differential and absolute in pregnant women at second trimester .

Parameter	Sample	No	Mean± Std	p.value
T,WBCs×10^9/L	No abortion	86	6.2 ± 2.3	0.00
	Abortion	34	7.7 ± 2.1	
Lymphocytes%	No abortion	86	33.9 ± 9.1	0.81
	Abortion	34	32.6 ± 47.7	
Neutrophils %	No abortion	86	59.2 ± 9.9	0.00
	Abortion	34	68.6 ± 6.9	
Absolute lymphocytes	No abortion	86	1.9 ± 0.4	0.9
	Abortion	34	1.9 ± 0.6	
Absolute neutrophils	No abortion	86	3.9 ± 1.9	0.00
	Abortion	34	5.4 ± 1.6	

Significane level at p.value $p<0.05$.

Table (3.6) Effect of absorption on platelets count and platelets indices in pregnant women at second trimester.

Test	Sample	No	Mean± Std	p.value
Platelets $\times 10^9$ /L	No abortion	86	238± 45.5	0.19
	Abortion	34	222± 64.4	
PDW	No abortion	86	13.8± 1.6	0.08
	Abortion	34	13.2± 2.0	
MPV	No abortion	86	9.4± 0.5	0.00
	Abortion	34	10.4± 0.8	

Significane level at p.value p<0.05.

Table (3.7) Effect of number of pregnancy on Hb , HCT, RBCs count and RBCs indices in pregnant women at second trimester.

Test	Sample	No	Mean± Std	p.value
Hb g/dl	<3	44	10.8 ± 1.6	0.30
	>3	36	10.5± 1.2	
HCT %	<3	44	34.9± 4.9	0.27
	>3	36	33.8± 3.7	
RBCs×10^{12}/l	<3	44	3.9± 0.6	0.20
	>3	36	3.8± 0.4	
MCV /fl	<3	44	87.4± 9.4	0.86
	>3	36	87.7± 6.1	
MCH /pg	<3	44	27.6± 2.8	0.32
	>3	36	26.9± 3.8	
MCHC /g/dl	<3	44	31.5± 1.9	0.80
	>3	36	31.4± 1.9	
RDWcv	<3	44	13.8± 2.6	0.18
	>3	36	13.2± 1.8	

Significane level at p.value p<0.05.

Table (3.8) Effect of number of pregnancy on T.WBCs count, differential and absolute in pregnant women at second trimester .

Parameter	Sample	No	Mean± Std	p.value
T,WBCs×10^9/L	<3	44	7.6 ± 2.1	0.79
	>3	36	7.5 ± 2.5	
Lymphocytes%	<3	44	31.2± 41.8	0.61
	>3	36	27.9± 9.1	
Neutrophils%	<3	44	68.3± 6.5	0.10
	>3	36	65.3± 9.5	
Absolute lymphocytes ×10^9 /L	<3	44	1.8± 0.3	0.20
	>3	36	2.0± 0.7	
Absolute neutrophil ×10^9 /L	<3	44	5.3± 1.7	0.53
	>3	36	5.1± 1.8	

Significane level at p.value p<0.05.

Table (3.9) Effect of number of pregnancy on Platelets count and Platelets indices in pregnant women at second trimester .

Parameter	Sample	No	Mean±Std	p.value
Platelets 10^9/L	<3	44	230± 70.4	0.99
	>3	36	230±58.0	
PDW	<3	44	13.5±2.3	0.33
	>3	36	13.1±1.3	
MPV	<3	44	9.7±1.1	0.09
	>3	36	10.0±0.7	

Significane level at p.value p<0.05.

Table (3.10)Effect of age on Hb , HCT, RBCs count and RBCs indices in pregnant women at second trimester .

Parameter	Sample	No	Mean± Std	p.value
Hb g/dl	<30	71	11.6± 1.8	0.04
	>30	48	11.0± 1.3	
HCT %	<30	71	36.9± 5.3	0.26
	>30	48	35.9± 4.4	
RBCs×10^{12}/L	<30	71	4.2± 0.6	0.18
	>30	48	4.8± 0.5	
MCV /fl	<30	71	87.1± 7.5	0.66
	>30	48	86.5± 6.5	
MCH /pg	<30	71	27.3± 3.3	0.61
	>30	48	27.1± 2.3	
MCHC /g/dl	<30	71	31.7± 2.0	0.06
	>30	48	31.1± 1.7	
RDWcv	<30	71	13.6± 1.1	0.51
	>30	48	13.3± 2.7	

Significane level at p.value p<0.05.

Table (3.11) Effect of age on T.WBCs count, differential and absolute in pregnant women at second trimester .

Parameter	Sample	No	Mean±Std	p.value
T,WBCs×10^9/L	<30	71	7.0± 2.5	0.06
	>30	48	6.2± 2.0	
Lymphocytes %	<30	71	29.8± 10.0	0.02
	>30	48	33.6± 8.4	
Neutrophils %	<30	71	62.9± 10.4	0.14
	>30	48	60.2± 9.6	
Absolute lymphocytes ×10^9 /L	<30	71	1.9± 0.5	0.87
	>30	48	1.9± 0.5	
Absolute neutrophils ×10^9 /L	<30	71	4.7± 2.1	0.01
	>30	48	3.4± 1.5	

Significane level at p.value p<0.05.

Table (3.12) Effect of age on Platelets count and Platelets indices in pregnant women at second trimester .

Parameter	Sample	No	Mean ± Std	p.value
Platelets ×10^9/L	<30	71	240± 55.7	0.21
	>30	48	226± 60.2	
PDW	<30	71	13.4± 1.9	0.03
	>30	48	14.0± 1.4	
MPV	<30	71	9.7± 0.8	0.54
	>30	48	9.8± 0.8	

Significane level at p.value p<0.05.

Chapter Four

Discussion, Conclusion and Recommendations

Chapter Four

Discussion, Conclusion and Recommendations

4.1 Discussion:-

The results of this study showed that There was significant decrease in Hb, HCT, RBCs count, lymph % and absolute lymph and PDW in pregnant women when compared with control group .and significant increase in,TWBCs neut% and absolute neut count in pregnant women when compared with control group. there was no significant difference in MCH, MCHC, Plets count ,MCV, RDWcv, and MPV in pregnant women when compared with control group . In previous study in west Bengal, India of hematological parameters in pregnancy the results showed that study group exhibited statistically significant lower values of Hb PCV, of pregnant women compared with the control .while WBC were significantly 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 neutrophil in both the study and control groups .in this present study lymphocyte counts were lower these results agreed with the results of my study. In other previous study, WBCs of pregnant women at third trimester increased insignificantly , while lymph decrease significantly than those women at first and 2^{nd} trimester when compared of different trimester with control the result was WBCs and Neut increased significantly ,but lymph was decrease significantly. Allso there was significant decrease in Hb, HCT, RBCs count, MCH and significant increase in TWBCs, Neut% , absolute Neut and MPV . There for MPV of pregnant women with history of abortion significantly increased compared to those with no history of abortion (Khalil,2012) . This result completely agreed with my current study .

In this study there was significant decrease in Hb and absolute Neut and significant increase in lymph % and PDW.also there was no significant difference in HCT, MCV, MCH, MCHC, RDWcv, TWBCs, Neut%, Plets count, MPV, RBCs count in pregnant women >30 years in age when compared with pregnant women <30 years in age.Pregnancy is commonly associated with thrombocytopenia due to poor nutritional intake may result in decreased intake of vitamin B_{12} 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 approximately 35% ,transfer of 300 mg of iron ot 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 10g/dl or the mean cell volume (MCV) is below 82FL (Hoffbrand *et al.*,2006).

4.2 conclusions:-

- The results of this study showed that There was significant decrease in Hb, HCT, RBCs count, lymph % and absolute lymph and PDW in pregnant women when compared with control group and significant increase in, TWBCs neut% and absolute neut count in pregnant women when compared with control group .
- There was significant decrease in Hb, HCT, RBCs count, MCH and significant increase in TWBCs, Neut% , absolute Neut and MPV.
- There was significant decrease in Hb and absolute Neut and significant increase in lymph % and PDW.

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 .

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Appendices

Appendix 1

Sudan University of Technology

College of Graduate Studies

Questionnaire to determain CBC of pregnant women in second trimester attended in Ommderman Locality

NO( )

Presonal data :

Name:……

Age:……

Occupation:……

Husband occupation:……

Month of pregnancy:……

NO of pregnancy:……..

Aborstion: yes ( ) how many time( ) No ( )

Supplementation intake yes( ) No ( )

Regular ( ) irregular ( )

Visit to cilinic: yes( ) No ( )

Suffer from other disease :

Malaria ( ) Anemia ( ) Typhooid (|)

Other:

Previous blood transfusion :…….....

Result:

WBC…… RBC……. HGB……

HCT…….	MCV……	MCH…..
MCHC…….	PLT…….	LYM%.....
NEUT%.....	MIX%.....	LYM#.....
NEUT#.....	MIX#.....	RDW…….
PDW……	MPV……	

Appendix 2

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكلنوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم ومبحث المناعه الدمويه

براءة اخلاقية

الاسم:.....

سوف يتم اخذ عينة من الدم (3مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة اخذ العينه بواسطة المطهر.

كل الادوات المستخدمه لاخذ العينة معقمة ومتبع فيها وسائل السلامه المعمليه .

اوافق انا المزكور اعلاه على اخذ عينة لاجراء الدراسة

الامضاء :.....

التاريخ :.....

Alqaesartravel @ gmail .com

Appendix 3

Principle of Sysmex KX.21N:-

Sysmex KX.21N the sysmex is hematology automated analyzer use to quickly perform full blood count and reticulocyte count .it is made by the sysmex corporation .Blood is sample and diluted and moves through atube thin enough that cell pass by one at time ,becaue not everything about the cells can be measured at the same time ,blood is separated into nmber of different channels .As the cells pass through apertures the signels are transmitted in sequence to analog circuit and then to particle size distribution analysis circuits for conversion to cumulative cell size distribution data particle size distribution curves constructed and the auto discrimination level is then set by the microproceor for each population, This floating threshold allow for discrimination of all population ,the cell count include the pulses between the lower and the upper auto discrimination level.

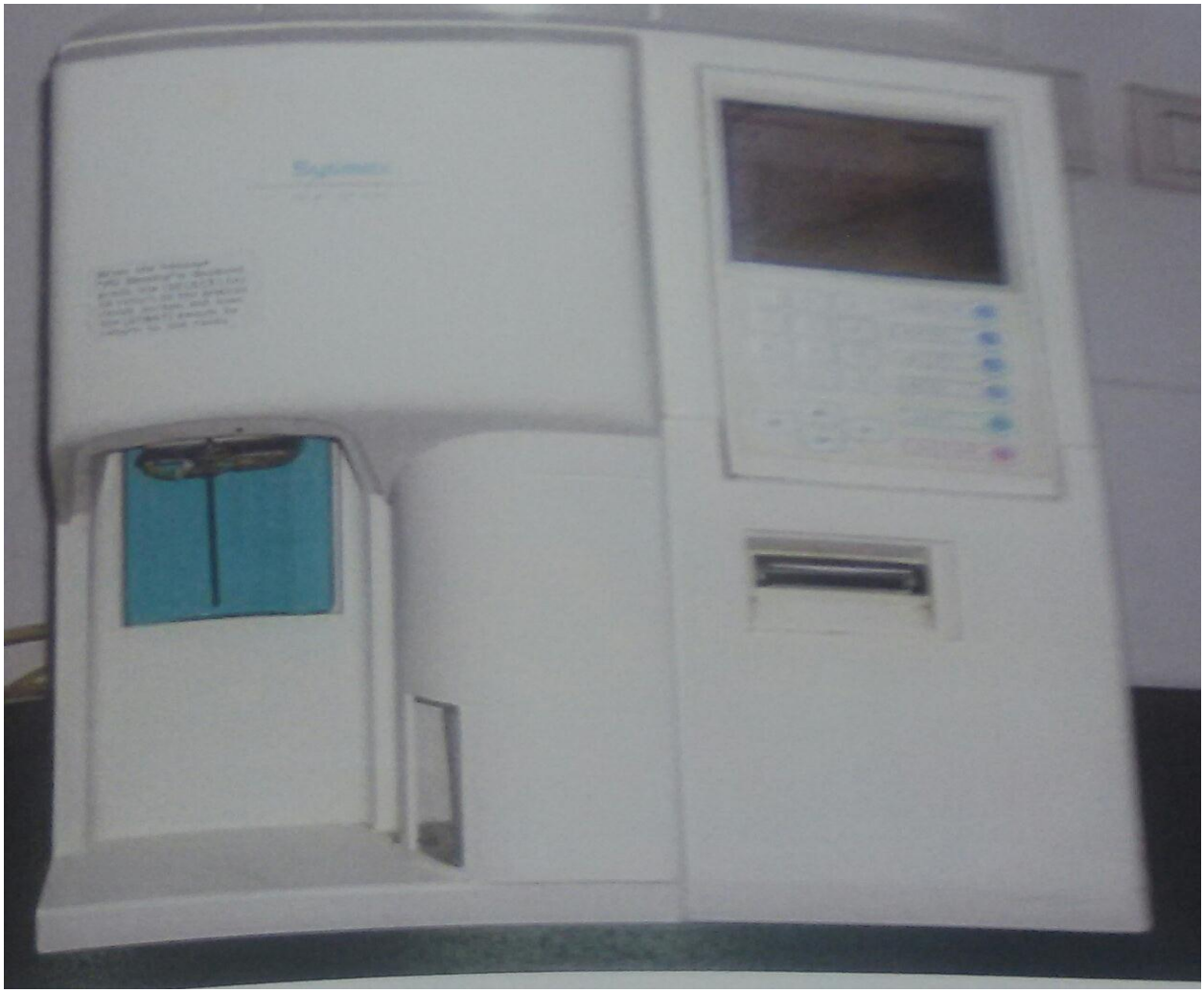

Figure 4 .1 Sysmex KX.21N