Sudan University of Science and Technology

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Measurement of Complete Blood Cells Count of Sudanese Pregnant Women at Third Trimester of pregnancy at Omdurman locality.

قياس تعداد الدم الكامل لدى السودانيات الحوامل في الثلث الاخير من فترة الحمل بمحلية ام درمان.

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الاية.

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صدق الله العظيم.

سورة القلم الاية رقم ﴿1﴾,﴿2﴾.

Dedication.

For you who were endured hardships for us……Father.

For you who is under your feet the paradise……..Mother.

My beloved husband who give me all compromise and support.

My teacher Khalada, Order all the love and respect for her.

To all who make the effort and contribution for me…my colleagous.

All my love for …… My sister God blesses you.

To all those who contributeted and helped to bring out this work

You have my full graduate and thanks.

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Abstract.

This is a case control study conducted during March to April 2015, at Omdurman locality in Military Hospital Obstetric Unit, to measure the complete blood cells count in Sudanese pregnant women at third trimester of pregnancy. Eighty pregnant women at the third trimester of pregnancy and Forty apparently healthy non pregnant women with matched ages were selected as control group. All participants were informed about research before collection of samples and informed consent was obtained from each. A questionnaire was designed to collect information about the study group as age, number of children, month of pregnancy, history and number of abortion, interpregnancy interval and supplementation intake. 2.5 ml venous blood was collected from each participant in k^2EDTA anticoagulant container. Automated hematological analyzer (Sysmex KX21N) was used to measure Complete Blood Count, and the results were analyzed by independent T test and one way ANOVA test of the SPSS computer programme.

The results of this study showed significant decrease in Hb (11.5±2.0 g/dl versus 12.2±1.1 g/dl), PCV (36.1±3.2% versus 37.9±3.4 %), RBCs count (4.1±.4×10^{12}/L versus 4.7±1.2 ×10^{12}/L), platelets count (237.9±59.8×10^9/L versus 276.3±61.1×10^9/L), lymphocytes percentage (24.2±9.4% versus 29.2±9.1%) of pregnant women compared to control group respectively. Signifcant increase in WBCs count (7.8±2.4×10^9/L versus 6.8±2.1×10^9/L), percentage of neutrophil (63.1±10.3% versus 59.2±16.1%), Absolute Neutrophil Count (5.3±2.7×10^9/L versus 4.3±1.9×10^9/L), Platelet Distribution Width (14.9±2.19 versus 12.8±2.4), Red cell Distribution Width - coefficient

of variation (14.6±1.8% versus 14.0±1.0%) of pregnant women compared to control group respectively. No significance differences in, Percentage of Absolute Lymphocyte Count, mixd cells, Mean Platelet Volume, Mean Cell Volume, Mean Cell Hemoglobin and Mean Cell Hemoglobin Concentration in test group compared with control group.

The study showed a significant decrease in Hb related to presence of history of abortion and significant decreased in Hb, MCV and MCH by increased the frequency of abortion. A significant decrease in Hb and PCV related to interpregnany interval and Irregular supplementation intake like iron and folic acid was observed and no significant variations in Hb level, PCV and RBCs count related to age groups and number of children.

The study was concluded: there was significant decrease in Hb, PCV, RBCs count, lymphocytes percentage and platelets count of pregnant women compared with control ($p< 0.05$)**,** significant increased in means of TWBCs and neutrophil's percentage and absolute, RDW-CV and PDW of pregnant women more than control ($p < 0.05$). No significant differences in Absolute Lymphocyte Count, Mean Platelet Volume , MCV, MCH, MCHC and percentage of mixed cells ($p> 0.05$).

مستخلص البحث

هذه الدراسة أجريت بطريقة الحالة الإفرادية المقترنة بحالة ضابطة في الفترة من مارس الى ابريل 2015. في ام درمان في المستشفى العسكري قسم النساء والتوليد لقياس صورة الدم الكاملة عند النساء الحوامل في الثلث الاخيرمن فترة الحمل. تمت مقارنة ثمانين عينة جمعت من نساء حوامل وفقا لنظام الإختيار المحدد من الشهر السادس الى التاسع من شهور الحمل بعد اخذ موافقتهن مع أربعين عينة من نساء متزوجات غير حوامل كعينات ضابطة. جميع المشاركين أعلم بالبحث و اهدافه قبل اخذ العينات واخذ من كل شخص الاقرار بالموافقة والمشاركة. تم اخذ 2.5ملي لتر عينة دم وريدية من كل متبرعة ووضعت فى إناء بلاستيكى يحتوى على مانع تجلط (k^2EDTA) وتم اختبارها لقياس صورة الدم الكاملة بإستخدام جهاز تحليل الدم الآلي (Sysmex KX21N). تم تحليل النتائج بإستخدام الفرق بين المتوسطين غير المعتدين فى برنامج الحزم الإحصائية للعلوم الإجتماعية المحوسب.

استنتجت هذه الدراسة الاتي : هناك انخفاض ذا دلالة احصائية في خضاب الدم (11.5±2.0 g/dl versus 12.2±1.1 g/dl)، الدم المكدس (36.1±3.2% versus 37.9±3.4 %) ، عدد كرات الدم الحمراء ($4.1\pm.4\times10^{12}$/L versus $4.7\pm1.2\times10^{12}$/L) ، عدد صفائح الدم ($237.9\pm59.8\times10^{9}$/L versus $276.3\pm61.1\times10^{9}$/L), النسبة المئوية للخلايا اللمفاوية (24.2±9.4% versus 29.2±9.1%) لدى النساء الحوامل عند مقارنتها بالعينات الضابطة بالتسلسل. هناك ارتفاع ذا دلالة احصائية في عدد كرات الدم البيضاء ($7.8\pm2.4\times10^{9}$/L versus $6.8\pm2.1\times10^{9}$/L). النسبة المئوية للخلايا العدلة (63.1±10.3% versus 59.2±16.1%), العدد المطلق للخلايا العدلة ($5.3\pm2.7\times10^{9}$/L versus $4.3\pm1.9\times10^{9}$/L), انتشار صفائح الدم (14.9±2.19 versus 12.8±2.4), انتشار الخلايا الحمراء (14.6±1.8% versus 14.0±1.0%) لدى النساء الحوامل عند مقارنتها بالعينات الضابطة بالتسلسل. لا يوجد اختلاف في العدد المطلق للخلايا اللمفاوية, متوسط حجم الخلية الحمراء، متوسط تركيز خضاب الدم في الخلية الواحدة، تركيز خضاب الدم في 100 مل من الدم و حجم صفيحة الدم الواحدة لدى النساء الحوامل وغير الحوامل.

إستخلصنا من هذه الدراسة ان هنالك انخفاض ذات دلالة معنوية في خضاب الدم , الدم المكدس ، عدد كرات الدم الحمراء ، النسبة المئوية للخلايا اللمفاوية عند النساء الحوامل مقارنة بالنساء غير الحوامل وزيادة ذات دلالة معنوية في عدد كرات الدم البيضاء، النسبة المئوية والعدد المطلق للخلايا العدلة , انتشار الخلايا الحمراء و انتشار صفائح الدم. عند النساء الحوامل مقارنة بالنساء غير الحوامل، هنالك انخفاض ذات دلالة معنوية في خضاب الدم عند النساء اللاتي تعرضن لإجهاض مقارنة باللاتي لم يتعرضن لاجهاض. وانخفاض ذات دلالة معنوية في خفاض الدم , متوسط حجم الخلية الحمراء و متوسط تركيز خضاب الدم في الخلية الواحدة بزيادة عدد مرات الاجهاض، أيضا هنالك انخفاض ذات دلالة معنوية في خضاب الدم , الدم المكدس عند النساء الاتي تبلغ الفترة بين الحملين من شهر الى سنه ونصف مقارنة باللاتي تبلغ الفترة بين الحملين اكثر من سنه ونصف وعند النساء اللاتي يأخذن مكملات كالحديد وحمض الفوليك بطريقه منظمه مقارنة بالاتي يأخذن مكملات كالحديد وحمض الفوليك بطريقه غير منظمه. لا يوجد اختلاف في خضاب الدم , الدم المكدس ، عدد كرات الدم الحمراء حسب الفئات العمرية وعدد الاطفال.

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Abbreviations:

ALC: Absolute Lymphocyte Count.

ANC: Absolute Neutrophil Count.

CBC: Complete Blood Count.

CO: Cardiac output.

CV: coefficient of variation.

DBC: differential blood count.

DM: Diabetes mellitus.

DNA: Deoxy nucleic acid.

DNC: differential neutrophil count.

DNC: differential lymphocyte count.

G6PD: Glucose 6 Phosphate Deficiency.

EPO: Erythropoietin

Hb: Hemoglobin.

HCT:hematocrit.

HiCN: cyanomethmoglobin method.

HTN: Hypertention.

K^2EDTA: Di- potassium Ethylene diamine tetra acetic acid.

LCD: Liquid Crystal Dialyzer.

MCV: Mean Cell Volume.

MCH: Mean Cell Hemoglobin**.**

MCHC: Mean Cell Hemoglobin Concentration.

MPV: Mean platelet volume.

PCV: Packed Cell Volume.

PDW: Platelet Distrubution Width.

PLt: Platelets.

RBCs: Red Blood Cells.

RDW: Red cell Distribution Width.

RNI: reference nutrient intake.

SPSS: Statistical package for social science.

SUST: Sudan University of Science & Technology.

TWBCs: Total White Blood Counts**.**

UK: United Kingdome.

UTI: Urinary Tract Infection.

WHO: World Health Organization.

Chapter one

Introduction and Literature review.

Chapter I

Introduction and Literature Review

1.1. Introduction:-

Pregnancy is the period from conception to birth. After the egg is fertilized by a sperm and then implanted in the lining of the uterus, it develops into the placenta and embryo, and later into a fetus. Pregnancy usually lasts 40 weeks, beginning from the first day of the woman's last menstrual period, and is divided into three trimesters, each lasting three months (Beck, 2009). Pregnancy is characterized by profound changes in nearly every organ system to accommodate the demands of the fetoplacental unit (Michael and Hossain, 2010). Normally, the uterus weighs 60 grams and is as large as a chicken egg. By the end of a pregnancy it will weigh 1 kilogram and contain a baby, a placenta and more than a quart of water. As the uterus grows it presses against the woman's abdominal organs. The uterus presses against the bladder, stomach and lungs, the arteries, veins and nerves and stretches the abdominal skin. These results in frequent urination, heartburn, congestion in the veins, difficulty of breathing and other conditions that will pass after birth as the uterus returns to its pre-pregnancy size. Pregnancy complications may cause both maternal death and fetal death if untreated. The most common pregnancy complications are ectopic pregnancy, Rh Negative Disease, infections, preterm labor, gestation diabetes, anemia, low birth (Haywood and Brown, 2013).

There are subtle and substantial changes in hematological parameters during pregnancy and the total blood volume increases by about 1.5 liter mainly to supply the needs of the new vascular bed. The most significant hematological changes are physiologic anemia, neutrophilia, mild thrombocytopenia, increased procoagulant factors, and diminished fibrinolysis (Elgari, 2013). Anemia during pregnancy is a major public health problem throughout the world, particularly in the developing countries. Anemia is defined as decreased hemoglobin level, or circulating red cells mass and is the most common hematological disorder during pregnancy (Singh *et al.*, 2014). This study is conducted to determine the hematological changes associated with pregnancy particularly, during the third trimester of pregnancy and is a part of a research program to establish data base for the reference values of some hematological parameters of Sudanese pregnant women. This data base provides researches with the information concerning CBC of Sudanese pregnant women at the third trimester of pregnancy period.

1.2. Literature review.

1.2.1. blood and Hemopoiesis:-

Blood is a vital intra vascular fluid circulates throughout the heart and blood vessels, and classified as connective tissue. Hemopoiesis is the process of production of blood cells (Hoffbrand *et al*., 2005). Blood compose of two portions, solid portion constituted (45%), consist of white blood cells, red blood cell and platelets. Fluid portion of plasma which constituted about (55%) (Hoffbrand *et al*., 2005).

1.2.1.1. Red blood cell:

An erythrocyte, a non-nucleated cell of the peripheral blood the main function of which is the transport of oxygen (Barbara and Rajeev, 2003). Contain hemoglobin, with a diameter = 8 μm, biconcave shape developmental period is 7 days, 120 days life span. (Berger *et al*., 2008).

1.2.1.2. Red cell indices:

Red cell indices a term which usually indicates: RBC, Hb, packed cell volume (PCV) which is a proportion of a column of centrifuged anticoagulated blood occupied by erythrocytes. The red cell indices provide information concerning the size and Hb content of red cells by providing the MCV, MCH, and MCHC. Mean cell volume (MCV) the average size of an individual's erythrocytes and is calculated by the formula as follows: MCV= (hematocrit/red cell count) x100 The normal value is between 80 and 100 fL and implies a red cell that has a size of 6 to 8 μm.Mean cell haemoglobin

(MCH) the average amount of haemoglobin in an individual's erythrocytes The MCH can be calculated by the following formula: MCH= (hemoglobin/red cell count) x100 The normal value is 27 to 31 pg, which implies that the average weight of Hb in a given amount of red cells is in the appropriate range. Mean cell haemoglobin concentration (MCHC) the average concentration of haemoglobin in an individual's Erythrocytes. The MCHC content can be calculated using the following formula (expressed in percentage):

MCHC= (hemoglobin/hematocrit)x10 The normal value is 32% to 36%, which impliesthat the amount of Hb per red cell is in the appropriate Concentration. (Ciesla, 2007).

1.2.1.3. Hemoglobin:

A complex molecule composed of four globin chains, each of which partially encloses a haem molecule, which has as its major function the transport of oxygen from the lungs to the tissues (Bain and Gupta, 2003).

1.2.1.4. Erythropoiesis:

Erythropoiesis (from Greek 'erythro' meaning "red" and 'poiesis' meaning "to make") is the process which produces red blood cells (erythrocytes) (Berger *et al.*, 2008).

1.2.1.5. White blood cells:

Cells of body defense which includes: Neutrophil granulocytes: defense against infections (particularly bacterial infections), Eosinophil granulocytes: relevant in allergic and parasitic diseases. Basophil granulocytes: relevant in allergic and parasitic diseases, Monocytes: resistance to infection and phagocytosis, B lymphocytes: antibody-mediated immune response and T

lymphocytes: cellular immune response. Granulopoiesis is formation of granulocytes (eosinophils, basophils, neutrophils. Lymphopoiesis is formation of lymphocytic effector cells (T lymphocytes, B lymphocytes). Myelopoiesis is formation of myeloid effector cells (granulocytes, monocytes, macrophages). (Berger *et al*., 2008).

1.2.1.6. Platelet:

Also known as "Thrombocytes," essential for coagulation and formation of clot in injurious tissues, size 1–2 μm, granular, basophilic, developmental period 10–12 days, life span of circulating thrombocytes 7–8 days. Thrombopoiesis Formation of thrombocytes (platelets) (Berger *et al*., 2008).

1.2.1.7. Haemopoietic growth factors:

A protein, often a glycoprotein, that promotes growth and differentiation of haemopoietic cells, e.g. erythropoietin, thrombopoietin (Bain and Gupta, 2003).

1.2.2. Anemia:

Anemia is a condition in which the volume of red blood cells is low. Red blood cells carry oxygen to cells throughout the body. Without oxygen, cells can't use the energy from food. Major causes of anemia include, Reduced production of red cells results from primary or secondary red cell hypoplasia or Aplasia, inadequate red cells formation of nutritional anemia's or hemoglobinopathies and Increased red cells break down results from shortened red cell life span in hemolytic anemia (Hoffbrand *et al*., 2005).

1.2.2.1. Symptoms of Anemia:

• Pallor of skin and mucous membranes, nail beds, conjunctivae.

• Weakness, tiredness, reduced performance.

• Lack of concentration, headache, and vertigo.

• Dyspnea, tachycardia, palpitations (esp. with acute anemia) (Dar, 2008).

1.2.2.2. Classification of anemia:

1.2.2.2.1. Morphological classification:

According to red cells morphology, hemoglobin level, hematocrit, red cell count, red cell indices (Dar, 2008). Include:

- ❖ Microcytic hypochromic anemias.
- ❖ Macrocytic normochromic anemias.
- ❖ Normocytic hypochromic anemias.

1.2.2.2.2. Etiological classification:

- ❖ Increase red cell destruction. Always causes red cell hemolysis.
  - ✓ Intracellular defect on red cell either in content or red cell membrane.eg: Thalassemia, sickle cell anemia, G6PD.
  - ✓ Extracellular factors, eg: Malaria infection, drugs action, Physical, Chemical and radiation and presence of antibodies against red cells.
- ❖ Increase blood loss.

  Either acute or chronic.
- ❖ Inadequetred cell production (Dar, 2008).

1.2.2.3. Diagnosis of anemia:

History, Clinical Examination:

• Risk factors like infections, drugs, bleeding (menstruation history), nutritional habits

• Clinical examination including skin, mucous membranes, lymph node status, spleen / liver findings, heart (tachycardia, particularly systolic murmur), rectal examination with fecal blood test, gynecological examination**.**

Laboratory Tests:

• Hematology: blood count, with MCV, MCH, reticulocytes, differential blood count, blood smear.

• Coombs' test (if hemolytic anemia is suspected).

• Virus serology (including parvovirus B19).

• Blood group.

• Eerythropoie.tin level (if renal anemia is suspected).

- Histology:

Bone marrow aspiration / biopsy, with iron stain (if stem cell/bone marrow disorder is suspected) (Berger *et al*., 2008).

1.2.2.4. Anemia of pregnancy:

The WHO definition of anemia in pregnancy is hemoglobin of less than 110 g/L but many laboratories will define their own pregnancy normal ranges that may be as low as 100 g/L (10 g/dL) (Singh *et al*., 2014). WHO has estimated the prevalence of anemia in pregnant women as 14% in developed and 51% in developing countries (65-70% in India). About one third of the global population (over 2billion) is anemic. It is estimated that about 60 million pregnant women worldwide are anemic. Only 4 million of these are in developed countries. Throughout Africa 50% of pregnant women are anemic (Singh *et al*., 2014). The consequences of anemia in pregnancy include: still-birth, low birth weight and pre-term births, reduced work capacity, decreased

mental performance, low tolerance to infections, death from anemic heart failure and maternal deaths due to uncontrolled bleeding . Third trimester of pregnancy is one of the factors associated with anemia (Obes *et al*., 2013). The Centers for Disease Control and Prevention has defined anemia in pregnancy as hemoglobin levels of less than 11 g/dL (hematocrit less than 33%) I n the first and third trimesters and less than 10.5 g/dL (hematocrit less than 32%) in the second trimester (Michael and hossin, 2010).Normally during pregnancy, the total blood volume increases by about 1.5 litters to supply the demands of the new vascular bed. The increase in plasma volume which leads to decrease hemoglobin concentration which lead to dilutional anemia (Chandra *et al*., 2012). To evaluate the genesis of anemia, the following laboratory values are taken into consideration when diagnose anemia in pregnancy:

*If anemia is from low iron the following is observed: -Microcytic/hypochromic red blood cells (smaller/paler than normal)

-Serum ferritin <11 ng/ml (mg/L)

-Transferrin saturation level <16%

-Serum iron <30 mcg/dl

-Mean corpuscular hemoglobin concentration (MCHC) <30 g/dl

-Iron-binding capacity increased (>400 mcg/dl)

*If anemia is from folic acid deficiency the following is observed:

-Enlarged red blood cells, meaning the MCHC will be increased (higher); this is directly opposite of iron-deficiency anemia (Derricott and Cartwright, 2013).

1.2.2.4.1. Iron Deficiency Anemia:

Most frequent form of anemia. Proportion male:female = 1:5. About 10–20% of women in childbearing age demonstrate latent iron deficiency. Daily iron resorption required: men 1 mg, women and adolescents 2–3 mg, pregnant women 3–4 mg. About 60–70% of body iron store bound in hemoglobin, additional 10% in myoglobin. 1 g hemoglobin contains 3.4 mg of iron. In iron deficiency the iron need is greater than the available iron supply, resulting in hemoglobin synthesis disorders → microcytic, hypochromic erythrocytes. (Berger *et al.*, 2008). Serum Ferritin: correlates with total iron is decreased in iron deficiency. serumTransferrin: correlates with circulating iron and need is decreased in iron deficiency. (Berger *et al.*, 2008).

Causes of Iron Deficiency:

• Poor iron uptake: infants, small children, vegetarians, alcoholics, nutritional disorders.

• Recommended daily uptake: men 12 mg, women 15 mg, pregnancy 30 mg.

• Defective resorption: postoperative (stomach resection), malassimilation

• Increased need: growth, pregnancy, lactation period, during treatment of vitamin B12 deficiency

• Blood loss: urogenital / gastrointestinal bleeding, cystitis, angiodysplasia, esophagitis, hemorrhoids

• Infection / parasites (worldwide most frequent cause of iron deficiency: hookworm infection) (Berger *et al.*, 2008).

Peripheral Blood:

Microcytic, hypochromic erythrocytes, poikilocytosis, anisocytosis, anulocytes. (Berger *et al.*, 2008).

Bone Marrow:

Iron stain (Prussian blue stain): storage iron not detectable (ferritin, hemosiderin). (Berger *et al*., 2008).

Symptoms of Iron Deficiency:

• Skin and nail changes: skin atrophy, spoon-shaped nails (koilonychia)

• Oral rhagades, impairment of mucous membranes, in extreme cases painful mucous membrane atrophy of tongue, pharynx, and esophagus with dysphagia (Plummer-Vinson syndrome). (Berger *et al*., 2008).

Laboratory Tests:

• Hematology: blood count, MCV ↓, MCH ↓, reticulocytes, differential blood count.

• Blood group (if red cell substitution necessary).

• Iron resorption test (if resorption deficiency is suspected).

- Histology:

In inconclusive cases eventually bone marrow aspiration / biopsy, including iron staining (Berger *et al*., 2008).

1.2.2.5. Megaloblastic Anemia:

Anemia with increased erythrocyte volume (MCV > 98 fl), usually caused by lack of vitamin B_{12} (cobalamin) and/or folic acid. Vitamin B_{12} Deficiency Anemia Incidence 5–10 cases/100,000 population/year, distribution male:female = 3:2, age peak 60 years. The reference nutrient intake (RNI) for vitamin B_{12} is 1 µg, with maximum daily absorption in the terminal ileum of 2–3 µg. "Intrinsic factor" (glycoprotein) is a prerequisite for vitamin B_{12} resorption. Functions of Vitamin B_{12} (Cobalamin) are:

• Cofactor in the synthesis of succinyl CoA, methionine, and tetrahydrofolic acid.

• In case of vitamin B_{12} deficiency:

DNA synthesis and fatty acid metabolism impaired, delayed nuclear maturation, normal cytoplasmic development, ineffective myelopoiesis, large cells with altered nucleus: plasma ratio (Berger *et al.*, 2008).

Causes of Vitamin B_{12} Deficiency:

• Most frequent cause: pernicious anemia (80% of cases): autoimmune atrophic gastritis with antibodies against gastric parietal cells (90% of cases) and/or antibodies against intrinsic factor (50% of cases).

→ Achlorhydria, intrinsic factor deficiency.

→ Decreased vitamin B12 resorption in the terminal ileum.

• Insufficient vitamin B12 uptake (strict vegetarians, alcoholics).

• Postoperatively (gastric resection, resection of the terminal ileum, blind loop syndrome).

• Vitamin B12 malabsorption, rare (Crohn's disease, scleroderma, amyloidosis).

• Infections / parasites (fish tapeworm, bacterial gastrointestinal infections) (Berger *et al.*, 2008).

Peripheral Blood:

Macrocytic hyperchromic erythrocytes, poikilocytosis, anisocytosis, hypersegmented granulocytes (right shift); in severe cases, granulocytopenia and thrombocytopenia (Berger *et al.*, 2008).

Bone Marrow:

Megaloblastic changes: ineffective left-shifted erythro-, thrombo-, and granulopoiesis, pronounced erythropoiesis with increased numbers of immature erythroid precursors (erythropoietic hyperplasia with megaloblastic

erythroblasts), giant band forms, immature megakaryocytes (Berger *et al*., 2008).

Megaloblastic anemia related symptoms:

• Pale skin and mucous membranes, icterus (due to intramedullary hemolysis).

• Weakness, fatigue, reduced performance, dyspnea on exertion.

• Difficulty concentrating, headache.

- In advanced cases: funicular myelosis: neuropathy caused by symmetrical damage of the posterior.

columns of the spinal cord, the corticospinal tract and peripheral nerves; motor abnormalities mainly affecting the lower extremities; staggering gait, ataxia, spastic paresis, impaired vision, psychological disorders.

• Type A gastritis.

• Trophic disorders of the skin and mucous membranes: Hunter's glossitis, etc.

• Sterility (gonad dysfunction), reversible (Berger *et al*., 2008).

Laboratory Tests:

• Hematology: blood count with MCV (↑), MCH (↑), reticulocytes (↓), differential blood count.

• Antibodies against gastric parietal cells and/or against intrinsic factor.

• Vitamin B_{12} serum level (normal: 200–900 pg/ml), folic acid serum level.

• Vitamin B_{12} absorption test (Schilling's test): oral administration of radioactive B_{12} intrinsic factor, determination of urinary vitamin B_{12}, comparison of vitamin B_{12} absorption / excretion with and without intrinsic factor.

• Blood group (if red cell transfusion is necessary).

• Gastroscopy: detection of chronic atrophic gastritis, exclusion of gastric carcinoma (incidence 3 times higher with chronic atrophic gastritis).

• Bone marrow aspiration / biopsy to confirm megaloblastic.

• Bone marrow aspiration / biopsy to confirm megaloblastic abnormalities

Other Causes of Macrocytosis.

• Alcoholism (most common cause of a macrocytic blood count).

• Hepatic disorders, severe hypothyroidism.

• Reticulocytosis, myelodysplasia, paraproteinemia.

• Cytostatic agents (antimetabolites, anthracyclines, anthracenediones, etc.).

• Pregnancy, neonates (Berger *et al*., 2008).

1.2.3.Pregnancy:

Pregnancy is the period from conception to birth. After the egg is fertilized by a sperm and then implanted in the lining of the uterus, it develops into the placenta and embryo, and later into a fetus. Pregnancy usually lasts 40 weeks, beginning from the first day of the woman's last menstrual period, and is divided into three trimesters, each lasting three months (Beck, 2009)**.**

Pregnancy is typically divided into three trimesters. The first trimester is from week 1 through 12 and includes conception. Conception is followed by the fertilized egg traveling down the fallopian tube and attaching to the inside of the uterus, where it begins to form the fetus and placenta. The second trimester is from week 13 through 28. The third trimester is from 29 weeks through 40 weeks. Pregnancy causes physiologic changes in all maternal organ systems; most return to normal after delivery. In general, the changes are more dramatic in multifetal than in single pregnancies. (Beck, 2009)**.**

1.2.3.1. Physiologica changes during pregnancy:

Pregnancy causes physiologic changes in all maternal organ systems; cardiovascular, hematologic, renal, gastrointestinal, endocrine most return to

normal after delivery. In general, the changes are more dramatic in multifetal than in single pregnancies, and also can be summarized by Cardiac output increases 30 to 50%, the increase in Cardiac output during pregnancy is due mainly to demands of the uteroplacental circulation. Total blood volume increases proportionally with Cardiac output. The increased blood volume includes a 45% to 50% increase in plasma volume and 20% to 30% increase in red blood cells**.** Since these percentages are not equal, the subsequent hemoglobin (HGB)/hematocrit (HCT) will reflect a normal physiologic anemia of pregnancy. Changes in renal function roughly parallel those in cardiac function and to accommodate increased metabolic and circulatory requirements. GFR increases 30 to 50%, peaks between 16 and 24 wk gestation, and remains at that level until nearly term, so we found frequent urination in pregnancy period (Haywood and Brown, 2013). Lung function changes partly because progesterone increases and partly because the enlarging uterus interferes with lung expansion. As pregnancy progresses, pressure from the enlarging uterus on the rectum and lower portion of the colon may cause constipation. GI motility decreases because elevated progesterone levels relax smooth muscle. Pregnancy alters the function of most endocrine glands, partly because the placenta produces hormones and partly because most hormones circulate in protein-bound forms and protein binding increases during pregnancy. Increased levels of estrogens, progesterone contribute to pigmentary changes include melasma, although exact pathogenesis is unknown. These changes (mask of pregnancy), darkening of the mammary areolae, axilla, and genitals and linea nigra (Haywood and Brown, 2013). Interpregnancy interval: The time interval between pregnancies or the length of time from the first delivery to the next conception. (James *et al*., 1995)

1.2.3.2. Hematological Changes in Pregnancy:

Normal pregnancy is characterized by profound changes in nearly every organ system to accommodate the demands of the fetoplacental unit. The most significant hematological changes are physiologic anemia, neutrophilia, mild thrombocytopenia, increased procoagulant factors,and diminished fibrinolysis (Chandra *et al*., 2012).

- Red blood cell:

Red blood cell mass begins to increase at 8–10 weeks of gestation and steadily risen by 20–30% (250–450 cc) above nonpregnant levels by the end of pregnancy in women receiving iron supplementation. 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 level increases by 50% in normal pregnancies and vary according to the presence of pregnancy complications. The increased plasma erythropoietin induces the rise in red cell mass, which partially supports the higher metabolic requirement for oxygen during pregnancy (Michael and Hossain 2010). Normally during pregnancy, the total blood volume increases by about 1.5 litters to supply the demands of the new vascular bed. The increase in plasma volume which leads to decrease hemoglobin concentration which lead to dilutional anemia (Chandra *et al*., 2012). A true anemia: HGB <11.5 g/dl and HCT <30%. (Derricott and Cartwright, 2013).

- Red cell indices:

Mean corpuscular volume decreases during pregnancy. Also Red cell indices decreased in pregnancy incase of iron deficiency but some time MCHC increased in case of folate deficiency (Michael and Hossain 2010).

- Iron status:

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 (Michael and Hossain 2010).

- Folic acid:

The increase in red cell mass also necessitates an increased folic acid requirement. In nonpregnant women, the daily folic acid requirement is 50–100 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. (Michael and Hossain 2010).

- platelet:

platelet counts remain in the normal nonpregnant range in most women during uncomplicated pregnancies, mean platelet counts of pregnant women may be slightly lower than in healthy nonpregnant women (Michael and Hossain 2010).

- 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**.** 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. (Michael and Hossain, 2010). WBC levels above 20–30,000 cells/mm3, or shifts in the differential, especially a larger percentage of bands/stabs appearing, or a sharp increase in WBC level is characterized as abnormal changes (Derricott and Cartwright, 2013).

- Blood coagulation:

Normal pregnancy is a prothrombotic state. Protein S activity and free protein S antigen decrease, increases in the complement 4b binding protein related to the hormonal changes of pregnancy, resistance to activated protein C increases in the second and third trimesters, Fibrinogen, factors II, VII, VIII, and X increase by 20–200%; von Willebrand factor also increases. Activity of the fibrinolytic inhibitors and Factors V and IX remain unchanged and factor XI levels decrease by 30% (Michael and Hossain 2010).

1.2.3.3. Complications of Pregnancy:

Complications of pregnancy are health problems that occur during pregnancy. They can involve the mother's health, the baby's health, or both. some of common maternal health conditions or problems a woman may experience during pregnancy are anemia, Urinary tract infections, Mental health problem, Hypertention, Gestational Diabetes Mellitus**,** Obesity and Weight Gain**,** Infections**,** Hyperemesis Gravidarum (Haywood and Brown, 2013).

-Anemia:

Anemia means lower than normal number of healthy red blood cells. (Hoffbrand *et al*., (2001). The consequences of anemia in pregnancy include: still-birth, low birthweight and pre-term births, reduced work capacity, decreased mental performance, low tolerance to infections, death from anemic heart failure and maternal deaths due to uncontrolled bleeding (Obes *et al*., 2013).

1.2.3.4. Hematological Changes occur at Third trimester of Pregnancy:

During pregnancy, the total blood volume increases by about 1.5 litters to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery, plasma rennin activity tends to increase resulting in systemic vasodilation.the maternal erythropoietin production will increased but relatively less compared with the increase in plasma volume which lead to decrease hemoglobin concentration thus there is dilutional anemia (Michael and Hossain, 2010).

- RBCs and Red cell indices:

The drop in hemoglobin typically by 1-2g/dl by the late second trimester and stabilizes thereafter in the third trimester. Red blood cell indices change little

or no change in pregnancy; Mean corpuscular volume decreases during pregnancy and averages 80–84 fL in the third trimester Michael and Hossain, 2010).

-White blood cells:

White blood cells count increased in pregnancy with the lower limit of the reference range being typically 6000/cumm. Leukocytosis occurs due to physiologic stress, lymphocyte count increases during the third trimester, generally there are neutrophilia in pregnancy (Michael and Hossain, 2010).

-Platelet:

Large cross sectional studies done in pregnancy of healthy women have shown that the platelet count is decreased during pregnancy particularly in the third trimester and termed as gestational thrombocytopenia due to hemodilution and partly due to platelet activation and accelerated clearance (Michael and Hossain, 2010).

- Mean Platelet Volume and platelet distribution width:

The mean platelet volume and platelet distribution width increase significantly and continuously as gestational advances. (Chandra *et al.*, 2012). Increased platelets turnover and consequently more immature platelets in the maternal circulation may explain why MPV is increased. (Juan *et al.*, 2011).

- Red cell Distribution Width:

Varying forms of anemia can cause an imbalance in red blood cell size variation (Red cell distribution width). Too many immature red blood cells

may be a sign of degenerative anemia; while too many small red blood cells could be a sign of anemia caused be iron deficiency.

(http://www.babymed.com/laboratory-values/red-cell-distribution-width-rdw-whole-blood-during-pregnancy).

1.3. Previous study:

Anaemia during pregnancy is a major public health problem throughout the world, particularly in the developing countries. The WHO definition of anemia in pregnancy is hemoglobin of less than 110 g/L (11 g/dL) but many laboratories will define their own pregnancy normal ranges that may be as low as 100 g/L (10 g/dL) (Singh *et al*.,2014). WHO has estimated the prevalence of anaemia in pregnant women as 14% in developed and 51% in developing countries (65-70% in India). About one third of the global population (over 2billion) is anaemic. The prevalence of anaemia during pregnancy is much higher and has far reaching consequences, especially the severe degrees of anaemia. It is estimated that about 60 million pregnant women worldwide are anaemic. Only prevalence of anaemia in pregnant women varies anywhere between 50-90% among different population groups. In contrast to this, 18 to 20% of pregnant women in developed countries are anaemic 4 million of these arein developed countries. In developing countries, mainly in Africa 50% of pregnant women are anaemic (Singh *et al*., 2014). In Latin America, prevalence of anaemia in pregnant women is about 40% (Singh *et al*., 2014). South Asia has highest prevalence of anaemia. In a steering committee report from India. In India 16% of maternal deaths are due to anaemia (Singh *et al*., 2014). Severe anemia with maternal hemoglobin below 6 g/dL has been associated with reduced amniotic fluid volume, fetal cerebral vasodilation, and

nonreassuring fetal heart rate patterns. Increased risks of prematurity, spontaneous abortion, low birth weight, growth restriction, and fetaldeath have also been reported. (Michael and Hossain, 2010).

Astudy conducted in Nigeria by Akingbola which revealed that pregnancy is characterised by lowest values of haemoglobin parameters in third trimester three and there are statistically significant differences between the WBC, platelet counts, RBC, PCT, and PDW of women between the three trimesters. (Akingbola *et al.*,2006).

In Sudan, mainly in Sea Ports corporation Hospital and Port Sudan Teaching Hospital (Obstetric department). Abdalsalam (2012) reported that the WBC of pregnant women at third trimester increased insignificantly (p.value = 0.08) while lymphocytes decreased significantly (p.value= 0.01) than those women at first and second trimesters. Neutrophils 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, 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 of pregnant women with history of abortion significantly increased compared to those with no history of abortion (p.value= 0.03) (Abdalsalam, 2012).

Elgari (2013) conducted study at Omdurman Al Saudi Maternity Hospital, The study revealed that there were significant decreased in RBCs count, hemoglobin and packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and platelets count of pregnant women compared to control (P value <0.05). TWBCs count was increased significantly (P. value < 0.050).

Al-tahir (2010) in Khartoum state reported that there were no significant differences in PCV, RBCs count, MCV, MCH, MCHC during second and third trimester of pregnancy. But there was significant decrease in Hb, PCV and significant increase in WBCs count.

1.3. Rational:

Normal range is required generally in heamatology. It is particularly important during pregnancy to mother and is useful data about hematological status of pregnant women to avoid complication that can occur to mother and baby.

1.4. Objectives:

General objective.

Measurement of complete blood cells count of sudanese pregnant women at third trimester of pregnancy (Omdurman locality).

Specific objectives:

1- to determine Hb, Hct, TWBCs, RBCs count, red cell indices, platelet count, MPV, RDW, PDW, Differential Neutrophil count, Differential lymphocyte count , percentage of mixd cell and absolute values of neutrophil and lymphocyte at third trimester of pregnancy compared to control group.

2- To associate age, history and number of abortion with some hematological parameters under study at third trimester of pregnancy.

3- To compare some hematological parameters under study to number of children, interpregnancy intervals and supplementation intake.

Chapter two

Material and Methods.

Chapter II

Materials and Methods

2.1. Study Design:

This is a case control study conducted during the period from 5 March to 15 April 2015 in Omdurman locality at Elselah Eltibi Military Obstetric Unit. The study aimed to determine complete blood count in Sudanese pregnant women at third trimester of pregnancy.

2.2. Study population:-

The study includes 80 pregnant women at the third trimester of pregnancy and 40 apparently healthy non pregnant women with matched age as control group.

2.3. Inclusion criteria:-

Pregnant women at third trimester of pregnancy period with different age groups attending Obstetric Unit at Military Hospital during the period from 5 March to 15 April 2015 .

2.4. Exclusion criteria:-

Pregnant women in first or second trimesters of pregnancy or pregnant women at third trimester with diseases that may affect CBC parameters.

2.5. Ethical consideration:-

- The research was approved at the level of Research Committee of collage of Medical laboratory Science.
- All participants were informed about the research before collection of samples and informed consent was obtained from each participant.
- Blood samples were analyzed immediately.

2.6. Sample collection:-

2.5 ml K^2EDTA venous blood sample was collected from each participant. Using sterile disposable syringe, 2.5 ml blood drained into K^2 EDTA container to perform complete blood count using automated hematological analyzer (Sysmex KX21N).

2.7. Method of sample collection:

Requirements:

Plastic K^2EDTA containers.

Sterile cotton.

Alcohol (70%).

Disposable syringes.

Tourniquet.

Procedure:

Test and control samples was collected by a tourniquet applied above elbow, and superficial antecubital forearm vein was identified. The skin was sterile with 70% ethanol and allowed to dry. Syringe needle inserted correctly into the vein, and 2.5 ml of blood samples were taken from the ante-cubital vein of the forearm, tourniquet was released, needle removed, and 2.5 ml blood was drained into K^2EDTA container , and mixed with anticoagulant gently for several times. (Dacie *et al*., 2006).

2.8. Samples blood processing:

Blood cells counts, hematocrit or packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration for study population were measured using an Automated Hematological Analyzer System model KX21N.

2.9. Complete blood count:

Complete blood count was performed by 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 (Dacie and Lewis, 2011). Which based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes erythrocyte, and platelet using three hydraulic systems for, WBC, RBC, platelet and hemoglobin and display the results on the Liquid Crystal Displayer (LCD) (Bernadette *et al*., 2000). 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) (Dacie and Lewis, 2011). Red cell distribution width (RDW) a mathematical calculation that indicate to the amount of anisocytosis (variation in size). 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 (Dacie and Lewis, 2011). 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. The mean platelet volume (MPV) is the average volume of individual platelets derived from the Plt histogram and the impedance platelet size distribution curve. Other platelet parameters platelet distribution width (PDW), which is a measure of platelet anisocytosis.(Dacie and Lewis, 2011).

2.9.1. Reagents and materials:

Commercial close system reagents were provided by sysmex KX21N consist of :-

1- Cell pack.

2- Stromatolyser.

3- Detergent.

4- Cell cleaner.

2.9.2. Procedures:

1- Two and half ml venous blood was collected from each participant into K^2EDTA container.

2- All blood samples must be mixed manually by inverting twenty times or left for 3-5 minutes on a mechanical mixture immediately before use.

3- Run the counter with diluents alone to check background count (i.e. electronic noise and particulate material in diluents solution). This should be within acceptable limits specified by the manufacturer.

4- Applying the blood container in vertical position near to sucking tip and give him order to sucking volume of blood.

5- Wait 5 minutes and release the result out by using the printer (Dacie and Lewis, 2011).

2.10. Study analysis:-

Will be analyzing by SPSS computer program, Version 14.0 was used compare means independent test and one way ANOVA test. The significant level set at ($P \leq 0.05$).

Chapter three

Results.

Chapter III

RESELTS.

This is a case control study conducted at Elselah Eltibi Military Hospital from 5 March to 15 April 2015 aimed to determine complete blood count in Sudanese pregnant women at third trimester of pregnancy. Eighty pregnant women in third trimester of pregnancy period were included. There were 48 pregnant women (60.0%) between 16-28 years old and 32 (40.0%) were with age between 29-40 years (Table 3-1).

The results of this study Showed in group (1) significance decrease in Hb, PCV, RBCs count and significance increase in RDW of pregnant women compared with control. In group (2) significance increase in WBCs count, and DNC, ANC, significance decrease in DLC and no significance differences in other study parameters of pregnant women compared with control. In group (3) significance decrease in Platelet count and significance increase in PDW (Table 3-2).

(Table 3-3) Showed no significant different in Hb level, PCV, RBCs count and red cell indices in pregnant women with age between 16-28 years compared to pregnant women with age between 29-40 years (P-value > 0.05).

(Table 3-4) significant increase in Hb level, MCV and MCH in pregnant women with no history of abortion when compared with pregnant women with history of abortion (P value < 0.05). But no significant variations in PCV, MCHC and RBCs count between two groups.

(Table 3-5) Significant decrease was observed in Hb level, MCV and MCH of pregnant women by increased the number of abortion (P value < 0.05).

(Table 3-6) Showed no significant difference in Hb level, PCV, RBCs count and red cell indices of pregnant women according to number of children (P value > 0.05).

Significant decrease in Hb level and PCV ((10.8±2.7 g/dl), (35.0±3.2 %) respectively) in pregnant women with interpregnancy interval between .1-1.5 years (p-value< 0.05) when compared with interpregnancy interval more than 1.5 years ((12.0±1.1) g/dl, (36.8±3.1)% respectively.) but no significant differences on RBCs count between the two groups (P value > 0.05). (Table 3-7).

(Table 3-8) Showed significant decrease in Hb level and PCV ((10.5±2.5 g/dl), (34.5±3.3 %) respectively) in pregnant women with irregular supplementation intake when compared with pregnant women with regular supplementation intake (Hb and PCV were (11.8±1.7 g/dl), (36.5±3.1 %) respectively) (P value < 0.05) but no significant different in RBCs count between the two groups (P value > 0.05).

Table (3-1) Distribution of Study population according to age groups.

Age Group/years	Frequency	Percentage%
16-28	32	40%
29-40	48	60%
Total	80	100%

Table (3-2) CBC of pregnant women compare with non pregnant.

Hematological Parameters		Pregnant women N=80	Non pregnant group N=40	P-value
Group 1	Hemoglobin Concs g/dl ±SD	11.5±2.0	12.2±1.1	0.039
	Packed cell Volume % ±SD.	36.1±3.2	37.9±3.4	0.006
	Red cell count $x10^{12}$/L ±SD.	4.1±.4	4.7±1.2	0.006
	Mean cell volume (fl) ±SD.	86.1±6.7	86.4±5.3	0.821
	Mean cell hemoglobin (pg) ±SD.	28.1±2.7	27.9±2.2	0.698
	Mean cell hemoglobin concentration (g/dl) ±SD.	32.5±1.6	32.3±1.2	0.359
	Red cell distribution width ±SD.	14.6±1.8	14.0±1.0	0.048
Group 2	White blood cell count $x10^9$/L ±SD.	7.8±2.4	6.8±2.1	0.029
	Percentage of neutrophil $x10^9$/L ±SD.	63.1±10.3	59.2±16.1	0.003
	Percentage of lymphocyte $x10^9$ /L ±SD.	24.29.4	29.2±9.1	0.006
	Absolute neutrophil count $x10^9$/L ±SD.	5.3±2.7	4.3±1.9	0.021
	Absolute lymphocyte count $x10^9$/L ±SD.	1.8±.7	1.9±.6	0.532
	Percentage of mixd cells$x10^9$/L ±SD.	7.9±2.4	7.9±2.2	0.970
Group 3	Platelet count $x10^9$/L ±SD.	237.9±59.8	276.3±61.1	0.001
	Mean platelet volume ±SD.	8.7±1.0	9.0±1.2	0.106
	Platelet distribution width ±SD.	14.3±2.4	12.8±2.4	0.002

Table (3-3) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to age interval/years old. (N=80).

Hematological parameters	No	Age interval from 16-28/years old N=48	Age interval from 29-40/years old N=23	P-value
wHeamoglobin g/dl ±SD	80	12.2±1.3	11.5±1.6	0.324
PCV % ±SD	80	36.8±3.2	36.3±4.2	0.668
RBCs count $x10^{12}$ ±SD	80	4.1±.4	4.2±.5	0.395
MCV(fL)	80	87.5±7.4	84.4±4.6	0.125
MCH (pg)	80	29.1±2.7	27.8±1.9	0.078
MCHC(g/dl)	80	33.1±1.0	32.9±.8	0.429
RDW-CV%	80	15.0±1.9	15.1±1.8	0.846

Table (3-4) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to history of abortion. (N=80).

Hematological parameters	No	Presence of history of abortion, N=24	No history of abortion, N=56	P-value
Heamoglobin g/dl ±SD	80	10.6 ±.3	11.9 ±1.3	0.052
PCV % ±SD	80	35.5±3	36.3±3.3	0.330
RBCs count $x10^{12}$ ±SD	80	4.2±.3	4.1±.5	0.312
MCV(fL)	80	83.4±6.7	87.2±6.4	0.020
MCH (pg)	80	26.8±2.9	28.6±2.5	0.006
MCHC(g/dl)	80	32.1±1.7	32.7±1.5	0.100
RDW-CV %	80	14.4±1.7	14.6±1.8	0.496

Table (3-5) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to number of abortion. (N=80).

Table (3-6) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to number of children. (N=80).

Hematological parameters	No	Pregnant women N=80			P-value
		No history of abortion N=56	History of abortion = 1, N=18	History of abortion >1 N=6	ANOVA
Heamoglobin g/dl ±SD	80	11.9±1.3	11.3 ± 1.4	10.5 ± 3.3	0.028
PCV % ±SD	80	36.3±3.3	35.8 ± 2.9	34.9 ± 3.3	0.530
RBCs count $x10^{12}$ ±SD	80	4.1±.5	4.2 ± .3	4.4 ± .3	0.325
MCV(fL)±SD	80	87.2±6.4	85.2±6.5	78.1±4.0	0.005
MCH (pg)±SD	80	28.6±2.5	27.3±3.0	25.1±2.0	0.005
MCHC(g/dl)±SD	80	32.7±1.4	32.0±1.8	32.2±1.6	0.256
RDW-CV %	80	14.6±1.9	14.6±2.5	14.6±2.4	0.724

Hematological parameters	No	Number of children 0-2,N= 58	Number of children more than 2, N=22	P-value
Heamoglobin g/dl ±SD	80	11.6±1.8	11.2±2.5	0.312
PCV % ±SD	80	36.2±3.3	35.7±3.1	0.499
RBCs count $x10^{12}$ ±SD	80	4.2±.4	4.1±.4	0.219
MCV(fL)	80	85.6±6.5	87.4±7.2	0.297
MCH (pg)	80	28.0±2.6	28.1±3.0	0.962
MCHC(g/dl)	80	32.7±1.2	32.1±2.1	0.185
RDW-CV%	80	14.5±1.7	14.5±2.0	0.913

Table (3-7) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to interpregnancy interval.

Hematological parameters	No	Interpregnancy interval between .1-1.5 years, N=31	Interpregnancy interval more than 1.5 years, N=49	P-value
Heamoglobin g/dl ±SD	80	10.8±2.7	12.0±1.1	0.022
PCV % ±SD	80	35.0±3.2	36.8±3.1	0.017
RBCs count $x10^{12}$ ±SD	80	4.0 ± .5	4.2 ± .4	0.061
MCV(fL)±SD	80	86.2±7.1	85.9±6.5	0.865
MCH (pg)±SD	80	27.8±3.0	28.2±2.6	0.620
MCHC(g/dl)±SD	80	32.2±1.7	32.7±1.4	0.138
RDW-CV%	80	14.8±1.9	14.4±1.7	0.325

Table (3-8) Hemoglobin, Hematocrit, RBC count, red cell indices and RDW of pregnant women according to supplementation intake. (N=80).

Hematological parameters	No	supplementation intake regular, N= 62	supplementation intake irregular, N=18	P-value
Heamoglobin g/dl ±SD	80	11.8±1.9	10.5±2	0.013
PCV % ±SD	80	36.5±3.3	34.4±3.1	0.017
RBCs count $x10^{12}$ ±SD	80	4.2±.6	3.9±.6	0.119
MCV(fL)±SD	80	86.0±6.6	86.1±7.1	0.990
MCH (pg)±SD	80	28.2±2.6	27.6±3.1	0.429
MCHC(g/dl)±SD	80	32.7±1.5	31.9±1.7	0.054
RDW-CV%	80	14.5±1.9	14.7±1.6	0.664

Chapter Four

Discussion, conclusion and recommendation.

Chapter IV

Discussion, Conclusion, and Recommendations

This study aimed to determine complete blood count of sudanease pregnant women at third trimester of pregnancy.

This study revealed that Hb level, PCV, RBCs count of pregnant women were significantly decreased (p-value<.05) compared with control group. This may be due to physiological changes associated with pregnancy which a reduction in hemoglobin concentration result from dilution due to the plasma volume expands more than the erythrocyte volume. The hematocrit in pregnancy normally drops several points below its pregnancy level (Williams and Wilkins, 2006). Pathological anemia is a common complication of pregnancy, occurring in approximately half of all pregnancies. In pathologic anemia of pregnancy, the oxygen-carrying capacity of the blood is deficient either due to decreased production of erythrocytes resulting from nutritional deficiency of iron, folic acid, or vitamin B_{12} or from sickle cell or another chronic disease, malignancy, chronic malnutrition, exposure to toxins or excessive loss of erythrocytes through destruction from inflammation, chronic infection, sepsis, autoimmune disease, microangiopathy, or a hematologic disease in which the erythrocytes are abnormal (Williams and Wilkins, 2006). The findings are consistent with previous previous studies in Khrtoum Teaching Hospital in Sudan by Al-tahir *et al* (2010) reported that there were significant decreased in Hb and PCV at second and third trimester of pregnancy when compared to pregnant women at first trimester**.** Elgari (2013) reported that there were significant decreased in RBCs count, hemoglobin and packed cell volume at pregnancy period when compared with control group.

RDW is significantly higher which may related to presence of anemia mainly iron deficiency anemia and folate deficiency anemia (Beck ,2009).

There were significant increase in white blood cell count, differential neutrophil count and absolute neutrophil count (p-value <.05) and significantly decrease in DLC when compared with control group. This finding in agreement with previous study in India which reported that total leukocyte count rising in early pregnancy and remained elevated through pregnancy. This may be as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, in the presence of a strong antimicrobial immunity. Similarly to the previous study conducted in India by Rouse *et al* (2011) reported that pregnancy leucocytosis, primarily related to increased circulation of neutrophils in the second month of pregnancy and Elgari (2013), which reported there was a neutrphilia during pregnancy. Abdalsalam (2012) a study in Sea Ports corporation Hospital and Port Sudan Teaching Hospital report that Lymphocytes percentage decreased significantly (p.value 0.01) in pregnant women at third trimester than those women at first and second trimesters of pregnancy.

There was significant decrease in platelet count of pregnant women compared to control which was in agreement with study reported that: platelet counts decreased in most women during uncomplicated pregnancies (Matthews *et al.*, 1990). Mean platelet counts of pregnant women may be slightly lower than in healthy non pregnant women (Verdy *et al.*, 1997). This partly may due to platelet activation and accelerated clearance (Chandra *et al.*, 2012). PDW also significantly higher than control group which may related to increase platelet activation, in agreement with study in Nigeria reported that: Only

PDW showed a significant increase from the first to the third trimester of pregnancy (P=0.009) (Vagdatli *et al*., 2010).

No changes in MCV, MCH, MCHC, and Absolute lymphocyte count in pregnant women compared with control. These findings are consistent with previous studies in Khartoum state by Al-tahir *et al* (2010) which reported that there were no significant differences in MCV, MCH, MCHC during second and third trimester of pregnancy. But is not consistent with previous studies by Elgari (2013) which reported The MCV, MCH and MCHC were significantly lower in pregnant women compared to non pregnant women. The difference may be due to using specific trimester of pregnancy period on current study.

The results of this study showed no significance different in Hb level, PCV, RBCs count red cell indices and RDW related to age groups, and number of children.

Significant decreased in means of Hb related to presence of history of abortion and a significant decreased in MCV and MCH related to history and frequency of abortion.

A significant decreased in means of Hb and Hematocrit related to decreased in interpregnany interval. Which are may be due to maternal serum and erythrocyte concentrations of folate decrease from the fifth month of pregnancy onwards and remain low for a fairly long time after delivery. Women who become pregnant before folate restoration is complete have a raised risk of folate insufficiency at the time of conception and during pregnancy. (Smits and Essed, 2001).

Significant decrease in hemoglobin related to irregular supplementation intake. The findings are consistent with previous studies (Sloan *et al*., 2002) Which reported that Iron supplementation raises hemoglobin levels.

Conclusions

1- Hb, PCV, RBCs count of pregnant women are significance decreased compared to non pregnant.

2- WBCs, percentage of neutrophil and ANC of pregnant women are significance increased and DLC was significantly decreased compared to non pregnant.

3- No significant difference in Hb, PCV, RBCs, red cell indices and RDW according to age interval or number of children.

4- Our study showed significant decreased in Hb according to presence of history of abortion and a significant decreased in Hb, MCV and MCH by increased the number of abortion.

5- There was a significant decreased in Hb and PCV of pregnant women with interpregnany interval between .1-1.5 years compared with pregnant women with interpregnany interval more than 1.5 years.

6- A significant decreased in Hb and PCV of pregnant women with irregular supplementation intake compared with pregnant women with regular supplementation intake.

Recommendation:

1- Regular measurement of complete blood cells count during late pregnancy period.
2- Regular follow up to detect their hematological status.
3- Regular intake of iron or folate supplementation were needed.
4- Blood film test is recommended to know the type of anemia in addition iron profile and folate assessment are necessary during pregnancy.

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Appendices.

Appendix (1).

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

دراسة لنيل الماجستير

اقرار بالموافقة والمشاركة

الاسم.....

سوف يتم اخذ عينة من الدم الوريدي بواسطة حقنة وذالك بعد تعقيم منطقة اخذ العينة بواسطة مطهر, جميع الادوات المستخدمة معقمة ومتبع فيها جميع وسائل السلامة العملية وليس هناك اثار جانبية للعملية ,قد يحدث تورم بسيط في منطقة اخذ العينة وسوف يزول بعد فترة قصيرة.الغرض من اخذ العينة هو البحث العلمي وسوف يتم تسليمكم نسخة من النتائج,وسوف تحفظ النتائج بسرية تامة .

أوافق انا المذكور اعلاه على اخذ عينة دم.

الاسم..... التوقيع.....

Appendix (2).

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Determination of Complete blood count of pregnant women at third trimester of pregnancy in Omdurman locality.

- **Sample number**…….

(1) Demographic data:

- Name: ……
- Age: ……
- No of Children…….
- Month of pregnancy:……..….
- Interpregnant interval……..
- Abortion: yes ( ),how many time ( ) No ( )
- No of abortion……..
- Supplementation intake: yes ( ) No ( )
  Regular( ) irregular( )
- Suffer from other disease:
  Malaria( ) Anemia ( ) Typhoid( )
- Other:…… …
- Previous blood transfusion: yes ( ),when( ) No ( )

(1) Hematological investigations:

Hb [g/dl]….. , PCV [%]….. , RBCs count [*10^12/L]….. , TWBCs [*10^9/L]….. , MCV [fl]….. , MCH [pg]….., MCHC [g/l]….. ,NEUT%..... ,MXD%.....,RDW%.....,PDW…..,MPV…..

Date. **Signature.**

Appendix (3)

Sysmex

Principle of Sysmex 21 Hematological Analyzer:

Measurement of blood cells (RBCs, WBCs and Platelet), and hemoglobin concentration were obtained by aspiration of small volume of well mixed blood with K^3EDTA by sample probe and mixed with isotonic diluents in nublazer. Diluted mixture aspiration delivered to RBC aperture bath for providing information about RBC and platelet based on cell size, were particles of 2 to 20 fl counted as platelets, above 36fl counted as red cells. Some portion of aspirated mixture was introduce into WBC bath in which hemolytic reagent (stromatolyzer) was added automatically to measure

hemoglobin concentration in build calorimeter, based on cyanomethmoglobin method (HiCN). Blood cells counted and size information generated in triplicate pulses according to electronic conductivity, and translated into digital number using in build calculator programmed and designed for that RBC, WBC counts, hence three values were directly measured (RBC, WBC, Hb), and displayed on (LCD). Other values of red cell indices, platelet counts, leukocyte differential and absolute count werecalculated from given information's and automated constructed histograms, the result were printed out according to the setting mode. (Bernadette *et al.,* 2000).