Sudan University of Science and Technology

College of Graduate Studies

Determination of Complete Blood Cell Count of Sudanese Pregnant Women at Second Trimester –Khartoum North Hospitals

قياس تعداد الدم الكامل لدي النساء الحوامل في الفترة الثانية من الحمل

بمستشفيات الخرطوم شمال

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

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قال تعالي:

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدق الله العظيم

سورة الإسراء الآية 85

Dedication

To:

My mother and My father

MyHusband

Son and Daughter

My brothers, sisters

My teachers

My friends

Acknowledgements

First all thanks to Allah for giving me the power and willing to complete this study. The words are unable to express my deep gratitude and sincere thanks and appreciation to my supervisor Dr.**KhaldaMirghaniHamza,** for her guidance, closes supervision, continuous follow up and her invaluable advices, and comments. I am indebted for her interest, constructive proposals and continuous encouragement. Thanks send to my teachers in theCollege of Medical Laboratory Science for their assistance and support. I wish to express my sincere gratitude and thanks to my family especially parents , husband for their love and support Thanks also extend to all staff in Hematology department for their assistance.

Abstract

This is a case control study, conducted in Khartoum North during the period from February to July 2015, to determine CBC (Hb, RBCs, HCT, MCV, MCH, MCHC, Platelets, WBCs, Leukocyte differential count, RDW, and MPV) of 80 Sudanese pregnant women at Second trimester as case and 40 non pregnant women as matched age as control. Pregnant women at second trimester were informed about the study and agreed for participation as case. A questionnaire was designed to collect information about the study group such as age, number of pregnancies, month of pregnancy, history of abortion .Two and half ml venous blood was collected in EDTA anticoagulant container. Automated hematological analyzer (Sysmex KX – 21N) was used to measure Complete Blood Count, and the results were analyzed by independent T test of the SPSS computerprogramme.

The results showedsignificant decreasein mean of Hct, TRBCs, Hb, MCHC and lymphocytes percentage($p \leq 0.05$) in pregnant women when compared withnon pregnant women.TWBCs, PDW, MPV and neutrophils percentage significantly increase($P = 0.00$). Insignificant decrease in means of MCV, MXD% and platelets in pregnantwomen(P.value$\geq$ 0.08)when compared withnon pregnant women. Insignificant increase in means of MCH and RDWsd(P.value$\geq$ 0.5). .

No significant difference in means of Hb, Hct, TRBCs count and indices and TWBCs count and differential ,platelets and their indices ($p > 0.13$), between with and without abortion .

There was no significant difference between means of Complete blood cell count in pregnant women($p>0.1$) who's had less or more than three children .

There was no significant difference between means of Complete blood cell count in pregnant womenwho's age more or less than 30 years.

In conclusion , Hct, TRBCs, Hb, MCHC and lymphocytes and neutrophil percentage,TWBCs, PDW, MPV significantly affected by pregnancy at second trimester.

المستخلص

اجريت هذه الدراسة بطريقة الحالات الإفرادية المقترنة بحالات ضابطة في الخرطوم شمال في الفترة من فبرايرالي يوليو 2015 لقياس صورة الدم الكاملة عند النساء الحوامل خلال الثلاثة أشهرالوسطي من الحمل. تم إختيار ثمانين من النساء الحوامل وفقا لنظام الإختيار المحدد وعوملن كعينات إختبارية بعد اخذ موافقتهن، وتم إختيار أربعين إمرأة من غير الحوامل وفقا لنظام الاختيار المحدد وعوملن كعينات ضابطة. تم اخذ 2.5

EDTA) . ملي لترعينه دم وريديه من كل متبرعه ووضعت في اناء بلاستيكي يحتوي علي مانع تجلط (

وتم اختبارها لقياس صورة الدم الكاملة بإستخدام جهاز تحليل الدم الآلي.ثم تحليل النتائج بإستخدام برنامج الحزم الإحصائية للعلوم الإجتماعية المحوسب.

اظهرت نتيجه هذه الدراسة ان هنالك نقص ذات دلالة معنوية في الدم المكدس, عدد كرات الدم الحمراء,خضاب الدم ,نسبه خضاب الدم في الخليه الحمراء والخلايا اللمفاوية عند النساء الحوامل مقارنة بالنساء غير الحوامل، وزيادة ذات دلالة معنوية في متوسط عدد كرات الدم البيضاء, متوسط حجم صفيحه الدم الواحده,انتشار الصفائح الدمويه والنسبه المئويه للخلايا العدله .نقص ذات دلالة غير معنوية في متوسط حجم الخليه الحمراء ,الخلايا المختلطه البيضاء وصفائح الدم. ، وزيادة ذات دلالة غير معنوية في تركيز خلايا الدم في الخليه الواحده ونسبه انتشار الخلايا الحمراء

لا يوجد اختلاف احصائي في اختبار الدم الكامل بين النساء الذين حدث لهم او لم يحدث لهم اجهاض. وكذلك لا يوجد اختلاف احصائي في قياس الدم الكامل للنساء الذين لديهم اقل اواكثر من ثلاثه اطفال.

لا يوجد اختلاف احصائي في اختبار الدم الكامل بين النساء الذين اعمارهم اقل او اكثر من 30 عاما.

Abbreviations

b- thalassemia beta thalassemia

CBC Complete Blood Count

CV Coefficient of variation DNA Deoxyribo nucleic acid

DIC Disseminated intra vascular co agulation

EDTA Ethylinediamine tetra acetic acid

flFemtolitter

g/lGram / litter

HELLP Hemolysis elevated liver enzyme and low platelet

HctHaematocrit

HSC Hemopoietic stem cell

IDA Iron deficiency anemia

IGg Immune globulin g

ITP Immune thrombocytopenic purpura

Kg killo gram

SD Standard Deviation

MCH Mean cell Hemoglobin

MCHC Mean cell Hemoglobin concentration

MCV Mean Cell Volume

MPV Mean Platelet Volume

NK Natural killer

PCV Packed cell volumepgPico gram

PDW Platelet distribution width

PltsPlatelets

RBC Red Blood Cell Count

RDWCVRed cell Distribution width by coefficient of variation

RDWSD Red cell Distribution width by standard deviation

RNA Ribo Nucleic Acid

SVR Systemic Vascular Resistance

SCD Sickle Cell Disease

WBC White Blood Cell Count

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Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

Pregnancy is the condition of having a developing embryo or fetus in the female body, after union of an oocyte (ovum) and spermatozoon. The average gestation period for a human pregnancy is 38 weeks from the first day of the last menstrual period (Abman and steven, 2011). Normal pregnancy is characterized by profound changes in almost every organ and system to accommodate the demands of feto-placental unit. In normal pregnancy , the physiological changes in hemoglobin concentration and platelet count during pregnancy are well known phenomena.It is also one of the physiological condition capable of causing remarkable and dramatic changes in hematological variable . Pregnancy is influenced by many factors, some of which include culture, environment, socioeconomic status , and access to medical care. The hematological indices of an individual to a large extent reflect their general health.Blood is a special type of connective tissue composed of formed elements in a fluid matrix. Anemia(low hemoglobin) is a widely identified hematological abnormality and it is also associated with adverse pregnancy out come .Anemia in pregnant women is variously defined with two common parameters either as hemoglobin concentration less than 11.0 g/dl or 5^{th} percentile of the hematocrit in a healthy referancepopulation.this study is possible through a series of tests measuring different variable of complete blood count(CBC) (James*et al.*, 2008).

The measure of CBC during pregnancy very important to follow up pregnant women , prevent anemia and inflammation during pregnancy ,there is no enough

studies about CBC in pregnant women ,this research illustrates the mean of all indices of CBC ,The effect of number of pregnancy and abortion on hematological parameters.

1.2 Literature review

1.2.1Blood

Blood is a bodily fluid in humans that delivers necessary substance such as nutrients and oxygen to the cells and transports metabolic waste prouducts away from those same cells ,when it reaches the lung, gas exchange occurs when carbon dioxide is diffused out of the blood into the pulmonary alveoli and the oxygen is diffused into the blood.Blood contains white and red blood cell,platelets,proteins, and other elements (Hormening, 2002) .The Blood function

- supplies tissue with oxygen (bounded to hemoglobin ,which carried in red cells)

-supply of nutrients such as glucose ,amino acid ,and fatty acid(dissolved in the blood or bound to plasma proteins(eg blood lipids)

-Removal of waste such as carbon dioxide ,urea ,and lactic acid

-Immunological function,including circulation of white blood cells,and detection of forign material by antibody

-Coagulation, the response to a broken blood vessel,the conversion of blood from aliquid to a semi solid gel to stop bleeding

-Messenger function,including the transport of hormones and the signaling of tissue damage

-regulation of body pH

-regulation of corebody temperature

-Hydraulic function

(Hormening, 2002) .

1.2.2 Hemopoiesis

1.2.2.1Red Blood cell

The most numerous cells in a blood are the red cells, also known as erythrocytes. Normal red cells are disc-shaped but are thinner in the centre As a consequence. Mature red cells in humans(although not in some other species) differ from most body cells in that they do not have a nucleus. Red cells are produced in the bone marrow and usually lose their nuclei when they are released into the blood stream(Bain, 2004).An average of 120 days, this soft and pliable cell moves with ease through the tissue capillaries and splenic circulation. As the cell ages, cytoplasmic enzymes are catabolized, leading to increased membrane rigidity (density), phagocytosis, and destruction.The term used to describe the process of erythrocyte production is erythropoiesis, Erythropoiesis encompasses differentiation from the hematopoietic stem cell (HSC) through the mature erythrocyte. Erythropoiesis epitomizes highly specialized cellular differentiation and gene expression. As cells progress through the stages of erythropoiesis, their potential to differentiate into lymphoid or other hematopoietic cell types is restricted. They are increasingly committed to differentiate into erythrocytes To streamline their functional capacity, erythrocyte precursors shed most organelles and produce prodigious amounts of hemoglobin, which eventually comprises approximately 95% of the total cellular protein. Erythropoiesis is regulated partially by the combined actions of cytokine signaling pathways and transcription factors.Hematopoiesis begins with the development of primitive erythrocytes in the embryonic yolk sac, continues in extramedullary organs such as the liver in the developing fetus, and is ultimately located in the red bone marrow during late fetal development, child hood, and adult life.Transport of oxygen to the tissues and transport of carbon dioxide from the tissues are accomplished by the hemepigment

in hemoglobin, which is synthesized as the erythrocyte matures. The basic substances needed for normal erythrocyte and hemoglobin production are amino acids (proteins), iron, vitamin B_{12}, vitamin B_6, folic acid (a member of the vitamin B_2complex), and the trace minerals cobalt and nickel. In adult humans, the daily production of more than 200 billion erythrocytes requires more than 20 mg of elemental iron. The vast majority of this iron comes from the recycling of senescent erythrocytes by macrophages of the mononuclear phagocytic system; only 1 to 2 mg of the daily iron supply derives from intestinal absorption, which at a steady state is sufficient only to replace iron lost by epithelial cell sloughing and functional and dysfunctional bleeding.Abnormal erythropoiesis can result from deficiencies of any of these necessary substances. Defective erythropoiesis is frequently seen in underdeveloped countries where protein deficiencies are common. Other types of anemias can be caused by deficiencies in vitamin B_{12}, folic acid, or iron(Turgeon, 2010).

1.2.2.1.1Hemoglobin

Human haemoglobin is formed from two pairs of globin chains each with a haem group attached. Normal adult hemoglobin (hemoglobin A) consists of four heme groups and four polypeptide chainswith a total of 574 amino acids. The polypeptide chains areorganized into two alpha chains and two beta chains. Each of the chains has an attached heme group. Normal adult hemoglobin has 141 amino acids in each of the alpha chains and 146 amino acids in each of the beta chains. The specific sequence of these amino acids is known and is important in the identification of abnormal hemoglobins involving substitutions of specific amino acids.In the native configuration of the hemoglobin molecule, the four hemes and four polypeptide chains are assembled in a very specific spatial configuration. Each of the four chains in the molecule coils into eight helices, forming an egg-

shaped molecule with a central cavity. In the process of the binding of the first heme group to a molecule of oxygen, a change in the overall configuration of the hemoglobin molecule occurs. This altered configuration of the molecule favors the additional binding of oxygen to the remaining heme groups, if sufficient oxygen pressure is present. Metabolic processes within the erythrocyte ensure a suitable intracellular environment for hemoglobin that protects it from chemical changes that might result in the loss of its native structure or denaturation. If hemoglobin is denatured, it loses its ability to carry oxygen (Turgeon, 2010).

1.2.2.1.2Hematocrit

The packed cell volume (PCV) can be used as a simple screening test for anaemia, as a reference method for calibrating automated blood count systems and as a roughguide to the accuracy of haemoglobin measurements. ThePCV×1000 is about three times the Hb expressed in g/l.In conjunction with estimations of Hb and RBC, it can be used in the calculation of red cell indices. However, its use in under resourced laboratories may be limited by the need for a specialized centrifuge and a reliable supply of capillary tubes (Bain, 2004).

1.2.2.1.3Red cell indices

Provide information about the hemoglobin content and size of red blood cells. Ab normal values indicate the presence of anemia and which type of anemia is it?

(Henry,2011) .

1.2.2.1.3.1Mean corpuscular volume

MCV is the average size of a red blood cell and is calculated by dividing the PCV by the red blood cell count

MCV= PCV/RBC×10

Normal range; 80-100 fL(Henrys,2011) .

1.2.2.1.3.2Mean corpuscular hemoglobin

MCH is the average amount of hemoglobin

MCH= HB/ RBC ×10

Normal range; 27-31 Pg(Henrys,2011) .

1.2.2.1.3.3Mean corpuscular hemoglobin concentration

Is the average concentration of hemoglobin per unit volume of red blood cell and is calculated by HB / HCT× 100

Normal range; 32-36%(Henry,2011) .

1.2.2.1 Red Cell distribution Width

Red cell distribution width is a measure of the variation of red blood cell volume that is reported as part of CBC usually red cellare standerd size of about 6-8 micrometer in diameter. higher RDW values indicate greater variation in size . Normal range is 11.5- 14.5 %. RDW can give of early change in RBCs which is a compined in iron deficiency anemia . women can benefited by doing CBC including RDW for the diagnosis of early iron deficiency anemia(Turgeon, 2010).

1.2.2.2 White Blood cells

In healthy people there are at least five types of white cell or leucocyte in the circulating blood. Unlike red cells, white cells have retained their nuclei. The cell is therefore made up of a nucleus and cytoplasm. The cytoplasm is the site ofprotein synthesis and other cellular functions. The nucleus is composed of chromatin, which is mainly deoxyribonucleic acid (DNA), carrying genetic messages. Genetic messages are transmitted from the nucleus to the cytoplasm by ribonucleic acid (RNA). White cells are divided into granulocytes (also known as

polymorphonuclear leucocytes) and mononuclear cells. There are three types of granulocyte and two types of mononuclear cell The names are not very logical but they have been in use for a long time and are generally accepted. Granulocytes are so named because their cytoplasm contains prominent granules. However, monocytes also have granules and so do some lymphocytes. The term polymorphonuclear leucocyte refers to the very variable nuclear shape which is typical of granulocytes. The term mononuclear cell means that the cell has only a single nucleus. However, this is true of granulocytes, as well as of the cells conventionally referred to as mononuclear (Bain, 2004).

1.2.2.2.1 Neutrophils

Neutrophils have a nucleus which stains purple and is divided into two to five segments or lobes. The lobes are separated by a thin strand or filament of nuclear material. The nuclear chromatin is heterogeneous with some clumping. The cytoplasm of neutrophils is very pale blue and is packed with fine lilac-staining granules. The granules are referred to asneutrophilic because they owe their colour to females aproportion of the neutrophils have a very small lobe, known as a‘drumstick’, protruding from the nucleus It represent the inactive X-chromosome of the cell. Neutrophils are produced in the bone marrow. They spend 6–10 hours in the blood stream before moving from capillaries into tissues. The major function of neutrophils is as tissue phagocytes. They move preferentially to sites of infection or inflammation where they ingest, kill and break down bacteria. The process of moving to sites of infection or inflammationis known as chemotaxis and occurs in response to activated complement components and chemical signals released by a variety of cells. The process of ingesting bacteria is known as phagocytosis (Bain, 2004).

1.2.2.2.2 Eosinophils

Eosinophils have a nucleus that is usually bilobed and pale blue cytoplasm, which is packed with large refractile,orange–red granules. The granules are referred to as eosinophilic because they take up the acidic dye eosin. Eosinophils are produced in the bone marrow and circulate in the blood stream forA normal basophil. The nucleus has three lobes. The cytoplasm ispacked with large purple granules. (The lower cell is a lymphocyte.)about 6 hours before migrating to tissues. They respond to chemotactic stimuli, are phagocytic and can kill ingested organisms. They are important in the body's defences against tissue parasites, being able to discharge their granule contentsextracellularly, seriously damaging large parasites. Eosinophils are also involved in allergic reactions (Bain, 2004).

1.2.2.2.3Basophils

Basophilshave a lobulated nucleus, which is often obscured by the large purple-staining granules which pack the very pale blue cytoplasm. The granules are referred to as basophilic because they take up basic components of the stain (such as methylene blue). In fact they stain metachromatically with basic stains, i.e. the granules react with a blue dye to produce a purple colour. Basophils are produced in the bone marrow and circulate in the blood in small numbers before migrating to tissue. They have a role in allergic and inflammatory responses (Bain, 2004).

1.2.2.2.4Lymphocytes

Lymphocytes are the second most numerous circulating whitecell after neutrophils. They are smaller than granulocytes witha round or somewhat irregular outline and pale blue, clear cytoplasm. Some lymphocytes have a variable number of azurophilic (pinkish-purple) granules. Lymphocytes are dividedinto three morphological categories, depending on their size, theamount of cytoplasm and the

presence or absence of cytoplasmicgranules. Small lymphocytes are most numerous. The nuclearchromatin of lymphocytes may be dense and homogeneous(particularly in small lymphocytes) Occasional normal lymphocytes show a discrete but ill-definedpaler structure within the nucleus, which is the nucleolus.Lymphocytes are produced from lymphoid stem cells in the bone marrow and probably the thymus. Their function is in tissues such as lymph nodes, spleen, tonsils and the lymphoidtissue associated with mucous membranes. They circulate in the blood stream, enter lymphoid tissues and emerge again from lymphoid tissues into lymphatic channels, where they form one constituent of a clear fluid known as lymph. Lymphatics drain into the thoracic duct and ultimately into the blood stream.This process of continuing movement between tissues and the blood stream is known as lymphocyte recirculation. Lymphocytes function in the body's immune responses. They are divided into three functional types: B cells, T cells and natural killer(NK) cells. B cells differentiate in tissues into plasma cells, which secrete antibodies, thereby providing humoralimmunity.T cells function in cell-mediated immunity as do(NK) cells. T cells also modulate B cell function. The functional categories of lymphocyte show little correlation with morphological categories except that large granular lymphocytes are either T cells or NK cells. However, other T cells cannot be distinguished morphologically from B cells. The functional categories of lymphocytes are of far more importance than the morphological categories(Bain,2004).

1.2.2.2.5Monocytes

Monocytes are the largest normal blood cells. They have lobulated nuclei and voluminous cytoplasm which is greyish-blue, is sometimes opaque and may be vacuolated or contain fine azurophilic granules. Monocytes have an intravascular life span of several days. They function mainly in tissues where they differentiate into long-lived macrophages (sometimes called histiocytes). Monocytes and

macrophages respond to chemotactic stimuli and are phagocytic. They are part of the body's defences against bacterial and fungal infections and also ingest and break down dead and dying body cells. They present antigen to lymphocytesThey secrete chemical messengers, known as cytokines, which influence the behaviour of other body cells, including blood cells and their precursors. Monocytes differentiate not only into macrophages but also into various specialized cells, specific to different organs, such as the Kupffer cells of the liver and the microglial cells of the brain(Bain, 2004).

1.2.2.3 platelets(Thrombocytes)

Platelets are produced within the vascular channels (sinusoids) of the bone marrow by the fragmentation of the protruding cytoplasm of large bone marrow cells known as megakaryocytes.They are thus not, strictly speaking, cells but rather are fragments of the cytoplasm of cells.Platelets are considerably smaller than red cells and white cells They are pale blue with fine azurophilic granuleswhich tend to be clustered in the centre of the platelet. When blood films are made, as is generally the case, from anticoagulated blood, the platelets are usually discrete and separate from each other, but in some circumstances they form clumps or aggregates (Bain,2004).

platelets Marrow transit time, or the maturation period of the megakaryocyte, is approximately 5 days.It is believed that platelets initially enter the spleen, where they remain for 2 days. Following this period, platelets are in either the circulating blood or the active splenic pool. At all times, approximately two thirds of the total number of platelets are in the systemic circulation, while the remaining one third exist as a pool of platelets in the spleen that freely exchange with the general circulation. A normal person has an average of 250×10^9 /L (range, 150×10^9 /L to 450×10^9/L) platelets in the systemic circulation. Platelet turnover or effective

thrombopoiesis averages 350×10^9 /L ± 4.3×10^9/L/day.The life span of a mature platelet is 9.0 days ± 1 day. At the end of their life span, platelets are phagocytized by the liver and spleen and other tissues of the mononuclear phagocytic system(Barbara ,2004).Platelet function in hemostasisPlatelets normally move freely through the lumen of blood vessels as components of the circulatory system. Maintenance of normal vascular integrity involves nourishment of the endothelium by some platelet constituents or the actual incorporation of platelets into the vessel wall. This process requires less than 10% of the platelets normally in the circulating blood. For hemostasis to occur, platelets not only must be present in normal quantities but also must function properly(Turgeon, 2010).

1.2.2.3.1 platelet distribution Width

Platelets distribution width median was 13.3%, with a reference range of 10-17.9% . among all indices PDWrecieving attention due to its usefulness for distinguishing between reactive thrombocytosis, thrombocytosis associated with myeloproliferative disorder (Turgeon, 2010).

1.2.2.3.2 Mean platelet volume (MPV)

The mean platelet volume (MPV) is derived from the impedance platelet size distribution curve. The MPV is lower than predicted when thrombocytopenia is caused by megaloblastic anemia or bone marrow failure an increase in MPV has been observed in patients at risk of and following myocardial infarctionand cerebral infarction. MPV is the average volume of individual platelets derived from the Plt histogram. Itrepresents the mean volume of the Plt population under the fitted Plt curve multiplied by a calibration constant, and expressed in femtoliters (Dacie and Lewis, 2011).

1.2.3 Pregnancy

Also known as gravidity or gestation is the time during which one or more off spring develops inside a women(pregnancy condition information,2013) . Amultiple pregnancy involvesmore than one off spring such as with twins(Wylie, 2005). Pregnancy can occur by sexual intercourse reproductive technology . it usually last around 40 weeks (10 lunar month)from the last menstrual period and end in child birth.this about 38 weeks after conception (Abman and Steven, 2011). an embryo is the developing off spring during the first 8 weeks following conception after which the term fetus is used untill birth .symptom of early pregnancy may include missed period ,tender breast ,nausea and vomiting ,hunger, and frequent urination. Pregnancy may be confirmed with apregnancy test .Pregnancy is typically divided into three trimester . The first trimester is from week one through twelve and include 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 the fetus and placenta .the first trimester caries the highest risk of miscarriage(natural death of embryo or fetus).the second trimester isfrom week 13th through twenty eighth.Around the middle of second trimester , movement of the fetusmay be felt .At twenty eight weeks,more than ninety percent of babies can be survive out side the uterus if provide high quality medical care . The third trimester is fromtwenty nine week through forty weeks .Prenatal care improves pregnancy out comes , this may include taking extra folic acid ,avoiding drug and alcohol ,regular exercise ,blood test ,and regular physical exmination . Complication of pregnancy may include high blood pressure of pregnancy ,gestation diabtes,, iron deficiency anemia and severe nausea and vomiting(pregnancy condition information,2013).

1.2.3.1Second trimester of pregnancy

The second trimester is for many women the easiest three month of pregnancy in it feel better and the energy is up to start planning for baby arrival.During the second trimester the baby is grow quickly between 18^{th} and 22^{nd} week of pregnancy the mother made an ultrasound so the doctor can see the baby is progressing. Also can see the sex of baby.Vernix and lanugo keep the fetus skin from chapping in the amniotic fluid .Most of the brain neurons present by 24 weeks and the fetus react to sound, the fetus become 30cm in length and 820gm in weight(Berk,2011).

1.2.3.2Third trimester of pregnancy

Seventh month there is a better chance that a fetus born during this month will survive. The fetus continues to grow rapidly, and may weigh as much as 3 lb (1.3 kg) by now. Now the fetus can look around its watery womb with open eyes. Eighth month growth continues but slows down as the baby begins to take up most of the room inside the uterus. Now weighing 4-5 lbs (1.8-2.3 kg) and measuring 16-18 inch (40-45 cm) long, the fetus may at this time prepare for delivery next month by moving into the head-down position. Ninth month adding 0.5 lb (227 g) a week as the due date approaches, the fetus drops lower into the mother's abdomen and prepares for the onset of labor, which may begin any time between the 37th and 42nd week of gestation. Most healthy babies will weigh 6-9 lb (2.7-4 kg) at birth, and will be about 20 inch long(Berk ,2011).

Hematological concentration of Hb value in pregnancy	
1^{st} trimester	124-135 g/ l
2^{nd} trimester	110-117 g/ l
3^{rd} trimester	106-109 g/ l

(DacieandLewis, 2012)

1.2.3.2 Physiological changes during pregnancy

-Bleeding gums; about half of pregnant women develop swollen,tender gums. Hormone change are send more blood to the gums ,making them to bleed more easily .the gum should go back to normal after the baby is born .

-Breast enlargement;thebrest growing as they prepare to feed the baby .

-Congestion and nose bleeds; hormonal changes cause the mucus membrane lining the nose to swell , which can lead to a stuffy nose and make the snore at night,these change can make the nose bleeding more easily .

-Frequent urination; uterus rise away from the pelvic cavity during te second trimester giving a brief break from having to keep going bathroom.

-Heart burnand constipation ; these are caused by the body make more of hormone calledprogesterone this hormone relaxes certain muscle ,including the ring of muscle of the lower esophagus that normally keeps food and acid down in the stomach (Guyton and Hall,2005).

-cardiovascular and hematologic changes, maternal circulation changes during pregnancy to accommodate an increase in blood volume of up to 50% .Due to the increase in work load ,a split first sound, asystolic murmur, or even aheartsound may be heard upon auscultation. The increase blood volume peaks in the third trimester and return to prepregnant state somewhere around 6-12 weekspost partum.The increase blood supply include a 45% to 50% increase in plasma volume and twenty to thirty% in red blood cell . Since this percentage are not equal, the subsequent hemoglobin /hematocrit will reflect a normal physiologic anemia of pregnancy . The HCT will appear to fall as the volume increases more than the packed cell count.During pregnancy ,the systemic vascular resistance

(SVR) of the blood vessels lowers due to increased level of hormone , these decreasing SVR is an expected result of the increasing progesterone and prostaglandin levels, which relax smooth muscle, produce vasodilation. As a result of the increase volume and decrease resistance, cardiac output rises. Therefore,there is anormal lowering of the blood pressure, especially in the second trimester. This sometimes causes dizziness or feeling faint in women as they rise to standing during the second trimester. Their pressure should stabilize and approach pre-pregnancy numbers by the third trimester. An abnormal rise in blood pressure could be an indication of preeclampsia , which involves multiple systems of the patient .White blood cell (WBCs): count especially neutrophil, increase normally during pregnancy. During active labor there may be anothernormalincrease ,even in the absence of infection . In non pregnant patients a normal WBC count is some where between 5 and 10 ,but for pregnancy those normal values can be between 6 - 16 (6000-16000cells/mm3) in the third trimester may reach 20 to 30 × 10^3cells/mm3 in labor and early postpartum. When evaluating for infection ,therefore, other clinical indicators-such as increase temperature ,bacteriuria, WBC in urine Uterine tenderness ,and fetal tachycardia should be check (Guyton and Hall,2005).

1.2.3.3Hormonal change during pregnancy

Hormone are chemicals that circulate in the blood , and they have powerful and varied function . Different hormones regulate body functions and carry massage from one part of the body to another .Hormone recognise emotional triggers:;they can cause you to cry when you are sad and to react to fear or danger with the fight or flight response .Hormone during pregnancy are there to help regulate the many changes taking place to enable your body to be born safely .Some of the most significant hormone in pregnancy are: Oestrogen ,progesterone ,

oxytocin,endorphins , prolactin. Hormone also play an important part in the process of birth(Rana, 2002).

1.2.3.4 Hematological changes during pregnancy

Pregnancy places extreme stress on the hematological system and an understanding of the physiological changes that result is obligatory in order to interpret any need for the therapeutic intervention .Physiological anemiaIs the term often used to describe the fall in hemoglobin concentration that occur during normal pregnancy Blood plasma volume increases by approximately 1250ml,or forty five percentage above normal by the end of gestation and although the red cell mass itself increasesby some twenty five percentage this still lead to fall in hemoglobin concentration .values below 10 g/dl are probably abnormal and require investigation (Hoffbrand*et al*.,2006).Type of anemia that include :-

1.2.3.4.1 Iron deficiency anemia

Iron is an essential element in the synthesis of hemoglobin. Iron deficiency is a common form of anemia. Although iron deficiency is awell defined category of anemia, confusing this type of anemia with other forms of anemia does occur. Diagnosing and treating an iron-deficient patient correctly are especially important in high-risk populations. The failure to do so can produce a significant public health problem.Etiology, although an individual's need for dietary iron is small and will only manifest itself after iron storage sites in the body have been depleted, IDA is one of the most frequently encountered types of anemias. Four pathophysiological categories can contribute to the development of IDA which may result from various categories with multiple conditions in each category .The major categories that result in IDA aredecreased iron intake. A deficiency of this type results when not enough iron is consumed to meet the normal, daily required

amount of iron (e.g., fad diets and an imbalanced vegetarian diet), Increased iron utilization. An increased demand for iron that is not met, such as during pregnancy, the growth years, or periods of increased blood regeneration, Excessive loss of iron (physiological or pathological iron deficiency). An excessive loss of iron can result from acute or chronic hemorrhage or heavy menstruation, Faulty or incomplete iron absorption (physiological iron deficiency). Conditions of faulty or incomplete iron absorption can be caused by achlorhydria in certain disorders or following gastric resection; or chronic diarrhea. If a gastroenterologic evaluation fails to disclose a likely cause of IDA, or in patients refractory to oral iron treatment, screening for celiac disease, autoimmune gastri tis, and Helicobacter pylori is recommended. Twenty to twenty-seven percent of patients with unexplained IDA have autoimmune gastritis, 50% have evidence of activeH. pylori infection, and 4% to 6% have celiac disease (Turgeon, 2010).

Iron deficiency may result from several other less commonly occurring conditions including a disorder of iron utilization, sideroblastic anemia; selected hemoglobinopathies; anemia related to chronic disorders; chronic inflammation; parasitic infections such as hookworm; and a deficiency of the plasma iron transporting protein, transferrin (Turgeon, 2010).Physiology

Humans have thirty five to fifty mg of iron per kilogram of body weight. The average adult has three and half to fivegram of total iron. Normal iron loss is very small, amounting to less than 1 mg/day. Iron is lost from the body through exfoliation of intestinal epithelial and skin cells, the bile, and urinary excretion. To compensate for this loss, the adult male has a replacement iron need of one mg/day. However, additional iron is needed during the growth years, pregnancy, and lactation. Some women require supplementary iron because of heavy menstrual blood loss.Operational ironconsists of iron used for oxygen binding and

biochemical reactions. In humans, most operational iron is found in the hemeportion of hemoglobin or myoglobin. Most operational iron is incorporated into the hemoglobin molecules of erythrocytes and is recycled. In normal adults, hemoglobin contains two thirds of the iron present in the bodyUp to six hundardmg iron is required for the increase in red cell mass and a further three hundard mg for the fetus .Despite an increase in iron absorption, few women avoid depletion of iron reserves by the end of pregnancy .compicated pregnancy ,the mean corpuscular volume (MCV)typically rises by approximately 4fl .A fallin red cell MCV is the earliest sign of the iron deficiency.later,the mean corpuscular hemoglobin (MCH) Falls and finally anemia result. Early iron deficiency is likely if the serum ferritin is below fifteen micro gram/L together with serum iron <10 micro mol / L and should be treated with oral iron supplementation . the use of routine iron supplementation in pregnancy is debated but iron is probably better avoided untill the Hb falls below ten g/dl or MCV below eighty twofl in the third trimester(Turgeon, 2010).

1.2.3.4.2 Megaloblasticanaemia of pregnancy

Megaloblasticanaemia during pregnancy results from an inadequate intake of folate to meet the increased requirements of pregnancy. A small proportion of cases are due to latent coeliac disease first becoming manifest during pregnancy. Rare cases are pernicious anaemia, although this disorder is uncommon in the child-bearing age group. The prevalence of megaloblasticanaemia of pregnancy varies in different populations, apparently depending on the nutritional status of the population. In well-nourished communities, but mild cases occasionally occur in spite of the widespread use of prophylactic folic acid.Folate deficiency,Folate requirements are increased approximately two fold in pregnancy and serum folate level fall to approximately half the normal range with the less dramatic fall in red cell folate .in the some part of the world ,megaloblastic anemia during pregnancy is

common because combination of poor diet and ex-aggregated folate is requirment.Given the protective effect of folate against neural tube defect,Folic acid four hundredmicro gram /day should be taken per conceptually and through out pregnancy .Food fortification with folate is now being practiced in many countries. it B_{12} deficiency is rare during pregnancy althoughserum vitamin B_{12} levels fall to below normal intwenty to thirty percent of pregnancies and low values are sometimes the cause of diagnostic confusion.Folic acid, 400µg daily, should be given as a supplement throughout pregnancy. In women who have had a previous fetus with a neural tube defect, five mg daily is recommended when pregnancy is contemplated and throughout the subsequent pregnancy. In women of childbearing age, a supplementary intake of folic acid of four hundredµg daily is recommended, so that this extra intake will be present from conception. Combined iron and folic acid preparations are generally satisfactory, except that they are usually expensive and, if the patient cannot tolerate theiron and stops taking the tablets, folic acid therapy will also bediscontinued(Hoffbrand*et al*., 2006).

1.2.3.4.3 Hemoglobinopathies in pregnancy

During pregnancy ,hemoglobinopathies,particularly sickle cell disease ,HB SC disease , Beta- thalassemia disease and alpha thalassemia , can worsen maternal and perinatal outcomes (for genetic screening for some of these disorders .Preexisting sickle celldiseaseSCD is a general term for abnormalities of hemoglobin structure, for example, hemoglobinopathies, in which the sickle gene is inherited from at least one parent. These genetic disorders are characterized by the production of Hb S, anemia, and acute and chronic tissue damage secondary to the blockage of blood flow produced by abnormally shaped red blood cells.Sickle cell anemia (Hb-SS), the most common form of hemoglobinopathy, is an expression of the inheritance of a sickle gene from both parents . Other sickle cell disorders result from the coinheritance of the sickle gene. Common variants

include Hb SC disease and b-thalassemia. Patients with this disease are living longer, new treatments are becoming available for adults as well as children, and early detection does matter. Almost every state in the United States screens the blood of all newborns for SCD.the sickle cell gene must be inherited from both parents. Hb S is different from Hb A because of a single nucleotide change (GAT to GTT) that results in the substitution of valine for glutamic acid at the sixth position on the beta chain of the hemoglobin molecule This results in abnormalities in polymerization (or gelation), with deoxygenation that leads to sickling. The end result of the polymerization is a permanently altered membrane protein. Two thirds of the red blood cells (RBCs) are removed by extravascular mechanisms.HB S C disease Sickle-cell Hb-C disease results from the inheritance of the Hb-S gene from one parent and the Hb C gene from the other. May first cause symptoms during pregnancy. The disease increases risk of pulmonary infarction by occasionally causing bony spicule embolization .Effect on the fetus are common but , if they occur , often include fetal growth restriction. Sickle cell-Beta thalassemia;The inheritance of the sickle gene from one parent and a b-thalassemia gene from the other results in the compound heterozygous state: sickle b-thalassemia . thedisorder is variable in its clinical manifestations but tends to be milder in blacks than in Mediterranean persons. Patients have moderately severe hemolytic anemia. Splenomegaly occurs in 70% of cases.Patients who are unable to produce any Hb AS beta thalassemiahave disease as severe as that of SS patients. Those with b thalassemia can make a small amount of Hb A and have less extensive hemolysis and vasoocclusive phenomena Beta thalassemia can be diagnosed by examining the blood film and through hemoglobin electrophoresis. The blood film reveals hypochromic, microcytic red cells with polychromatophilia, target cells, stippling, and, rarely, sickled cells. Hemoglobin electrophoresis

reveals that sixtytoninty percent of the hemoglobin is S and ten to thirty percent is fetal (F). The therapy is the same as for SS disease. Splenectomy may be beneficial if the spleen is sequestering red cells in significant amounts. Hb S/b-thalassemia is less severe than SCD. The spleen remains functional, but retinopathy is more commonIs similar to HBS_C disease but is lesscommon and more benign(Turgeon, 2010).

1.2.3.4.4 Thrombocytopenia

The platelet count typically falls by approximately 10%in an un complicated pregnany , in approximaely7%of women this fall is more severe and can result in thrombocytopenia (platelet count $<140\times10^9$/L.In over75% of cases this is mild and of un known cause, acondition referred to as incidental thrombocytopenia of pregnancy .approximately 21% of cases are secondary to hypertensive disorder and 4%are associated with immune thrombocytopenic purpura(ITP).Incidental thrombocytopenia of pregnancy;this is adiagnosis of exclusion and is usually detected at the time of delivery(Hoffbrand*et al.*, 2006) . The platelet count is always $>70\times10^9$/L and recovers with insix weeks. No treatment is required and the infant is not affected.Thrombocytopenia of hypertensive disorders ;this is variable in severity but the platelet count rarely falls to $<40\times10^9$/ L.Itis more severe when associated with pre-eclampsia and if severe the primary treatment is as rapid delivery as possible. The platelet count falls for a day or two after delivery and then recovers rapidly.The HELLP syndrome (hemolysis, elavated liver enzymes and low platelet) is a subtype of this category.Idiopathic thrombocytopenic purpura in pregnancy ,ITPrepresnts a particular problem ,both to the mother and fetus, as the antibody crosses the placenta and the fetus may become severly thrombocytopenic.Like all adult, pregnant women with ITP andplatelet count less than 50 do not usually need treatment .Treatment is required for women with

platelet count less than 10 who in their second or third trimester or who are bleeding treatment is with steroid , intravenous immuno globulin(IgG) and splenectomy as appropriate.At delivery,umbilical vien blood sampling or fetal scalp vein sampling to measure the fetal platelet count may be offered although their exact role in un clear .in general, caesarean section is not indicated when the maternal platelet count is less than 50 unless the fetal platelet count is known to be less than twenty . platelet transfusion may be given to mother in labour with very low platelet count or who are actively bleeding .New born of mothers with ITPshould have ablood count measured for the first 4 days of life as the platelet count may progressively drop. Acount greater than fifty is reassuring .cerebral ultra sound may be performed to look for intracranial hemorrhage .in new born without evidence of ICH , treatment with intravenous IgGis appropriate if the infants platelet count is less than twenty. Neonates with thrombocytopenia and ICH should be treated with steroids and intravenous IgGtherapy(Hoffbrand*et al.*, 2006).

1.2.3.4.5 Hemostasis and thrombosis

Pregnancy leads to ahyper coaguable state with consequent increased risks of thromboembolism and disseminated intravascular coagulation (DIC) .there is an increased in risk of thromboembolism and DIC .There is an increase in plasma factor VII,VIII and X, and fibrinogen and fibrinolysis is suppressed .these changes last for up to months into the puerperal period and the incidence of thrombosis during this period is increased . there is an association between thrombophilic conditions in the mother and with recurrent fetal loss. This is presumed to result from placental thrombosis and infraction(Hoffbrand*et al.*, 2006).

1.2.3.5 Blood transfusion

Is generally the process of receiving blood product into ones circulation intravenously.Transfusion are used for various medical condition to replace lost component of the blood. Early transfusions used whole blood ,but modern medical practice commonly uses only component of the blood , such as Red blood cell,white blood cell, plasma ,clotting factor ,and platelets.There are two primary reasons to need abloodtransfusionwhile pregnant .these include the development of severe anemia close to your due date ,or hemorrhaging at some point during the pregnancy. Most often ,the transfusion required for pregnant and recently pregnant women only involves red blood cells.it is less likely platelets and plasma will be needed . in order to receive the transfusion(Turgeon, 2010).

1.2.4 Previous study

1.2.4.1 Previous study in the World

In west BenegalIndia state previous study of hematological parameters in pregnancythe results showed that study group exhibited statistically significant lower values of hemoglobin, PCV of pregnant women compared with the control ($p< 0.05$). while WBC were significantly elevated. There was no significant difference in all hematological parameters among the three trimesters. The value of neutrophil is higher in the studied group than the control group, but there is no statistical difference between the value of neutrophil in both the study and control groups. lymphocyte and monocyte counts were lower in studied group than in controlwhile the eosinophil was significant higher in studied group than the control group (Das *et al.*, 2013).

1.2.4.2 Previous study in Africa

This examination was a cross sectional study of274 pregnant women who registered to attend the lagos university teaching hospital or lagos state university teaching hospital,nigeria, antenatal clinics between their first and third trimester. Blood(4.5ml) was collected from each participant into a tube containing the anti co agulantethylenediaminetetraacetic acid (EDTA). A full blood count was performd on each sample and the result were analyzed.Over all, the values obtained were (mean ±standard deviationSD):hematocrit level, 30.16%± 5.55%;hemoglobin concentration, 10.94± 1.86g/dl ; white blood cell , $7.81\pm2.34\times10^9$; platelets, 228.29± 65.6 $*10^9$: cell volume 78.30 ±5.70 fl, corpuscular hemoglobin ,28.5±4.8 pg, and corpuscular hemoglobin concentration ,36.45± 1.10g/dlWhen grouped by trimester , in second trimester the mean S± D value of packed cell volume ,29.76%± 5.21%. hemoglobin concentration values were 10.81 ±1.72 g/dl .

A statistically significant relationship was found to exist brtween packed cell volume and white blood cell count with increase in gestitional age (p=0.010 and 0.001, respectively). However ,there was no statistically significant association between platelet count and increase in gestation age (p=0.296) (Akinbami*et al*., 2013).

1.2.4.3**Previous study in Sudan**

This is a cross-sectional case control descriptive analytical study , conducted at Port Sudan 2012. The result indicated that RBC, Hematocrit and lymphocyte of pregnant women decreased significally(p.value 0.00,0.01,0.00 respectively) while MCHC increased significantly (p.value0.03)compared to control. WBC and Neutrophil increased significantly (p.value 0.00) . Hemoglobin, platelets, Eosinophils ,basophil and MPV decreased in significantly (p.value 0.05) while

MCV,MCH and RDW increased in significally (p. value >0.05) Hemoglobin ,MCV ,MCH and platelet of pregnant women with previous pregnancies between 7 to 10 pregnancies insignificantly decreased (p.value .0.05) compaed to other group .WBC of pregnant women with number of pregnancy between 1-3 pregnancies increased in significantly (p.value>0.05) compared with those of pregnancy between 4-7 and 7-10. WBC of pregnant women at third trimester increased in significally (p.value 0.08) while lymphocytes decreased significally (p.value0.01) than those women at first trimester and second trimester neutrophil of women at third trimester increased significantlycompared to those in first trimester and second trimesters (p.value 0.02).when compared of different trimester with control the result was WBC and Neutrophil increased significantly at third trimester (p.value0.00) but 4 lymphocyte was decrease significantly at third trimester (p.value 0.00), MPVof pregnant women with history of abortion significantly increased compared to those with no history of abortion (p.value 0.03),RBcs and HCTof pregnant women increase significantly (p.value 0.01) with regularly visits to clinics(79%)while basophil decreased in significantly (p.value>0.05) compared to those with ir regular visits clinics (21%) (Khalil, 2012).

1.3. Rationale

The rationale for conducting this research in pregnant women at second trimester is to advance knowledge in the medical conditions in pregnant women, association with physiological and hematological changes .Screening of hematological studies of Sudanese pregnant women is needed to detect these hematologicalchanges . Published data about pregnant women is few , so this study was conducted to add new and recent data about hematological studies of Sudanese pregnant women.

1.4. Objectives

1.4.1. General objective

To determine complete blood cell count of Sudanese Pregnant women at the Second trimester –Khartoum North.

1.4.2. Specific objectives

- To determine CBC of Sudanese Pregnant women at the second trimester in compare to non pregnant women.

-To compare between means red blood cells count and its indices , platelets count and its indices and WBCs in test and control groups according to abortion.

-To compare CBCbetween numbers of children on pregnant women at thesecond trimester.

Chapter two

Materials and Methods

Chapter Two

Materials and Methods

2.1 Study design:

This is a case control study conducted in period from January to June 2015 to determine CBC in pregnant women at Second trimester attendedKhartoum North Hospitals.

2.2 Study population:

One hundred and twenty Sudanese women ,80 as study group and 40 non pregnant as control in Khartoum North.

2.3 Inclusion criteria:

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

2.4 Exclusion criteria:

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

2.5 Ethical consideration:

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

2.6 Data collection:

Data was collected using questionnaire which was specifically designed to obtain demographic and clinical data.

2.7 Sample collection

Two and half ml venous blood was collected from individuals under study and dispensed in di Sodium EDTA container for CBC determination.

2.8 Procedure of complete blood count

Automated hematological analyzer (Sysmex KXN-21)was used to measure complete blood count, an appropriate blood sample is presented to the instrument and usually measure from 8 to 20 components for the basic CBC and white blood cell differential.Impedance counting systems depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors. (Dacie and Lewis, 2011).

2.8.1 Red blood cell count (RBC) and Platelet count

Red cells and other blood cells were counted in systems based on aperture impedance technology. Platelets can be counted in whole blood using the same techniques of electrical detection as is used for counting red cells. An upper threshold is needed to separate platelets from red cells and a lower threshold is needed to separate platelets from debris and electronic noise. RBC normal range in women $4.3 \pm 0.5 \times 10^{12}$/L, and platelet normal range in women $280 \pm 130 \times 10^{9}$/L (Dacie and Lewis, 2011).

2.8.2 Hemoglobin concentration (HB)

Automated counters measurehaemoglobin concentration by a nonhazardous chemical, such as sodium lauryl sulphate, imidazole, sodium dodecyl sulphate or dimethyl lauryl amine oxide, which avoids possible environmental hazards from disposal of large volumes of cyanide containing waste. Modifications include alterations in the concentration of reagents and in the temperature and pH of the

reaction. A non-ionic detergent is included to ensure rapid cell lysis and to reduce turbidity caused by cell membranes and plasma lipids. Measurements of absorbance are made for hemoglobin measurement at various wavelengths depending on the final stable haemochromogen, cyanmethaemoglobin, oxyhaemoglobin, methaemoglobinormonohydroxyferriporphyrin and at a set time interval after mixing of blood and the active reagents but before the reaction are completed.(Dacie and Lewis, 2011).

2.8.3Packed cell volume and Mean cell volume

Automated blood cell counters estimate PCV/haematocrit by technology that has little connection with packing red cells by centrifugation.automated instruments, the derivations of the RBC, PCV and MCV are closely interrelated. The passage of a cell through the aperture of an impedance counter or through the beam of light of a light-scattering instrument leads to the generation of an electrical pulse, the height of which is proportional to cell volume. The number of pulses generated allows the RBC to be determined,. Pulse height analysis allows either the MCV or the Hct to be determined. If the average pulse height is computed, this is indicative of the MCV and the Hct can be derived by multiplying the estimated MCV by the RBC. Similarly, if the pulse heights are summated, this figure is indicative of the Hct and the MCV can, in turn, be derived by dividing the Hct by the RBC. PCV Women normal range 0.41 ± 0.05 L/L and MCV Women normal range 92 ± 9 fl (Dacie and Lewis, 2011).

2.8.4 **Total white blood cell count (WBC)**

Total white blood cellis determined in whole blood in which red cells have been lysed. The lytic agent is required to destroy the red cells and reduce the red cell stroma to a residue that causes no detectable response in the counting system. The

following fluid is satisfactory: Cetrimide 20 g,10% formaldehyde (in 9 g/l NaCl) 2 ml, Glacial acetic acid 16 ml, NaCl 6 g, and water to 1 liter. Residual particles in a diluted blood sample are counted after red cell lysis. Normal range 4 - 10 × 10^9 L (Dacie and Lewis, 2011).

2.8.5 Automated differential count

Automated blood cell counter have a differential counting capacity, providing a three-part differential count. Counts are performed on diluted whole blood in which red cells are either lysed or are rendered transparent. A three-part differential count was categorized leucocytes as WBC-small cell ratio (equivalent to lymphocytes), WBC-middle cell ratio (equivalent to monocytes, eosinophils and basophils) and WBC-large cell ratio (equivalent to neutrophils). Normal differential count neutrophils 40- 80 %, lymphocytes 20-40 %, monocytes 2-10 %, eosinophils 1-6 %, basophils < 1-2% (Dacie and Lewis, 2011).

2.9Statistical analysis

Data were entered into computer and analyzed by SPSS used independent test .P value significant level ≤ 0.05.

Chapter three

Results

Chapter three

Results

This study was carried out at Khartoum North during the period from February to July 2015 to measure CBC of pregnant woman at the second trimester.

There was a significant decrease in mean of Hct, TRBCs, Hb, MCHC and lymphocytes percentage in pregnant womenwhen compared with non pregnant women and, significant increased in means of TWBCs, PDW, MPV and neutrophils percentage of pregnant women more thannon pregnant women .Insignificant decreased in means of MCV, MXD% and platelets in pregnant womenwhen compared withnon pregnant women . Insignificant increased in means of MCH andRDWsd of pregnant womenmore than non pregnant women at second trimesterwere detected.

No significant difference in means of Hb, Hct, TRBCs count and indices and TWBCs count and differential ,platelets and their indices , between with and without abortion in second trimester.

There was no significant difference between means of CBC in pregnant women who's had less or more than three children .

There was no significant difference between means of CBC in age of pregnant women more or less than 30 years.

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

parameter	Sample	No	Mean±sd	P value
Hb g/dl	Pregnant	80	11.4±0.8	0.00
	Non pregnant	40	12.4±0.6	
Hct %	Pregnant	80	34±2.3	0.00
	Non pregnant	40	36.6±2.2	
TRBCs $\times 10^{12}$ /l	Pregnant	80	3.9±0.3	0.01
	Non pregnant	40	4.2±0.4	
MCV fl	Pregnant	80	85.9±6.5	0.08
	Non pregnant	40	89.3±6.6	
MCH pg	Pregnant	80	29.6±2.5	0.70
	Non pregnant	40	29.5±2.1	
MCHC %	Pregnant	80	34±1.7	0.03
	Non pregnant	40	33.5±1.1	
RDWSD%	Pregnant	80	43.1±2.7	0.5
	Non pregnant	40	42.8±2.6	

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

parameter	Sample	No	Mean±Standard Deviation	P value
TWBCs $\times 10^9$ /l	Pregnant	80	7.0±2.3	0.00
	Non pregnant	40	5.1±1.2	
Lymphocyte %	Pregnant	80	24.6±5.8	0.00
	Non pregnant	40	32.9±8.5	
Neutrophil %	Pregnant	80	65.7±7.0	0.00
	Non pregnant	40	57±8.0	
MXD %	pregnant	80	9.6±3.0	0.2
	Non pregnant	40	10.3±2.8	

Table (3.3)Comparisonof platelet count and indices) Compare between pregnant women and non pregnant women

parameter	Sample	No	Mean±standard deviation	P value
Plt $\times 10^9$ /l	Pregnant	80	233±54.4	0.1
	Non pregnant	40	248±56.1	
PDW %	Pregnant	80	10.9±2.3	0.00
	Non pregnant	40	8.8±1.2	
MPVfl	Pregnant	80	9.0±1.3	0.00
	Non pregnant	40	7.8±1.1	

Table (3.4) Effect of previous history of abortion onHb, Hct, RBCs count and indices in pregnant women

parameter	Sample	No	Mean± standard deviation	P. value
Hb g/dl	yes	25	11.5±0.8	0.64
	no	55	11.4±0.7	
Hct %	yes	25	34.5±2.4	0.15
	no	55	33.7±2.1	
TRBCs $\times 10^{12}$ /l	yes	25	3.9±0.2	0.68
	no	55	3.9±0.3	
MCV fl	yes	25	85.5±6.1	0.75
	no	55	86.0±6.7	
MCH pg	yes	25	29.3±2.7	0.47
	no	55	29.8±2.4	
MCHC %	yes	25	34.1±1.7	0.96
	no	55	34.0±1.8	
RDWSD%	Yes	25	43.7±2.8	0.16
	No	55	42.8±2.6	

Table (3.5)Effect of previous history of abortion on TWBCs count, differential and absolute in pregnant women

parameter	Sample	No	Mean± standard deviation	P value
TWBCs $\times 10^9$ /l	yes	25	7.4±2.4	0.33
	no	55	6.8±2.2	
Lymphocyte %	yes	25	24.4±5.2	0.84
	no	55	24.7±6.0	
Neutrophil %	yes	25	65.7±7.6	0.95
	no	55	65.6±6.9	
MXD%	yes	25	8.9±3.3	0.14
	no	55	10.0±2.8	

Table (3.6) Effect of previous history of abortion onplatelet count and indices in pregnant women

parameter	Sample	No	Mean± standard deviation	P value
Plt $\times 10^9$ /l	yes	25	226±52.2	0.49
	no	55	236±55.6	
PDW %	yes	25	10.8±2.4	0.72
	no	55	11.0±2.2	
MPVfl	yes	25	8.8±1.4	0.25
	no	55	9.1±1.2	

Table (3.7)Effect of number of children on Complete Blood count in pregnant women

parameter	NO 2	N	Mean ± sd	P.Value
WBC×10^9 /l	>3	35	7.0±2.1	
	<3	45	7.0±2.4	0.9
HB g/dl	>3	35	11.4±0.7	
	<3	45	11.5±0.8	0.5
RBC×10^9 /l	>3	35	4.0±0.3	0.3
	<3	45	3.9±0.3	
HCT%	>3	35	33.8±2.3	0.6
	<3	45	34.1±2.2	
PLT×10^9 /l	>3	35	228±46.9	
	<3	45	236±59.8	0.5
MCV fl	>3	35	85±6.4	0.3
	<3	45	86.598	
MCH pg	>3	35	29.3±2.4	
	<3	45	29.9±2.6	0.2
MCHC%	>3	35	33.9±1.7	0.4
	<3	45	34.2±1.8	
Neutrophil %	>3	35	65.5±7.6	0.8
	<3	45	65.800	
LYMOCYTE %	>3	35	24.6±5.6	0.9
	<3	45	24.578±5.9	
MXD %	>3	35	9.4±3.1	0.5
	<3	45	9.8±2.9	
RDWsd %	>3	35	43.2±2.6	0.6
	<3	45	43.0±2.7	
PDW %	>3	35	10.9±2.0	0.8
	<3	45	11.0±2.5	

MPV fl	>3	35	9.0±1.3	
	<3	45	9.1±1.3	0.7

No2: Number of children
N: Number of pregnant women

Table (3.8)Comparison of Complete Blood count between pregnant women according to age

parameter	age	N	Mean ± sd	P.Value
WBC×10^9 /l	<30	50	7.0± 2.3	0.8
	>30	30	7.1±2.1	
HB g/dl	<30	50	11.6±0.8	0.1
	>30	30	11.3±0.7	
RBC×10^9 /l	<30	50	3.9±0.31	0.3
	>30	30	3.9±0.3	
HCT%	<30	50	34.2±2.3	0.3
	>30	30	33.7±2.1	
PLT×10^9 /l	<30	50	233±55.9	0.9
	>30	30	236±59.8	
MCV fl	<30	50	86±6.4	0.8
	>30	30	85±6.6	
MCH pg	<30	50	29.3±2.4	0.6
	>30	30	29.5±2.6	
MCHC%	<30	50	34.9±1.7	0.2
	>30	30	33.2±1.6	
Neutrophil %	<30	50	65.5±6.6	0.6
	>30	30	66±6.8	
LYMOCYTE %	<30	50	24.6±5.3	0.5
	>30	30	24.5±6.4	

MXD %	<30 >30	50 30	10.1±3.1 8.9±3.9	0.09
RDWsd %	<30 >30	50 30	43.2±2.6 42.7±2.8	0.9
PDW %	<30 >30	50 30	10.9±2.6 11.0±1.8	0.6
MPV fl	<30 >30	50 30	9.0±1.4 9.1±1.1	0.7

Chapter four

Discussion, Conclusion, and Recommendations

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4.1. Discussion

This study designed to provide means and difference between healthy pregnant women and control(healthy women without pregnancy) of hematological parameters at second trimester in Khartoum North.

Pregnancy causes significant changes in metabolism ,fluid balance,organ function and blood circulation which are driven by estrogen and the presence of the feto – placental unit. These dramatic changes influence a wide variety of hematological parameter(Elgari,2013).

In the present study There was a significant decrease in mean of Hct, TRBCs, Hb, MCHC and lymphocytes percentage($p \leq 0.05$)in pregnant womenwhen compared with non pregnant women. In a previous studyin west Benegal(India),the results showed that study group in second trimester Hb in(g/dl) 9.1±1.2,PCV(%) 32.4±4.3 , lymphocyte % 40.5 ±19.3. There were significant difference in hemoglobin concentration,PCV,,Lymphocyte ($p \leq 0.05$) (Das *et al.*, 2013), These results agreed with the results of the current study.In other previous study in sudan there was a significant decrease in HCT,HB,TRBCs, MCHC (Elgari, 2013)also These results agreed with the results of the current study.The decrease in hemoglobin and PCV may be due to increase in plasma volume during pregnancy causing haemodilution, hormonal changes that increase fluid retention and iron deficiency (Wahed, 2008).

In the present study There were significant increase in means of TWBCs, PDW, MPV and neutrophils percentage($P = 0.00$) of pregnant womencompared with non pregnant women.. The findings in agreement with previous studywhich revealedthat total leukocyte count increases in early pregnancy and remained elevated through pregnancy(Elgari,2013).Akinbami*et al.*, (2013)showed

WBC7.4±2.7×10^9/l, neutophil 47.9±17.9%.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(Rouse *et al*.,1998).

In the present study there wereInsignificant decrease in means of MCV, MXD% and platelets in pregnantwomen(P.value≥ 0.08)when compared with non pregnant women.(Elgari,2013) and Akinbami*et al*., (2013).These results agreed with the results of thisstudy except in platelet and MCV that showed significant decrease in previous study but in significantly decreased in present study. The decrease in plateletsapproximately twenty one percent of cases are secondary to hypertensive disorder and four percent are associated with immune thrombocytopenic purpura(ITP)(Hoffbrand*et al*., 2006).

In the present study There wereInsignificant difference in means of MCH andRDWsd(P.value≥ 0.5)of pregnant womenmore than non pregnant women.These result contrast to result of (Elgari,2013) who show significant decrease in mean of MCH.

In the present study There wereno significant difference in means of Hb, Hct, TRBCs count and indices and TWBCs count and differential ,platelets and their indices ($p > 0.13$), according to history of abortion . In previous study MPVof pregnant women with history of abortion significantly increased compared to those with no history of abortion(Khalil, 2012).These result contrast to result of presnt study.

In the present studyThere was no significant difference between means of Complete blood cell count in pregnant women($p > 0.1$) who's had less or more than three children .In previous study there were in significant decreasd in HB,MCV, MCH and platelet of pregnant women with previous pregnancies between 7 to 10 pregnancies compare to other group (Khalil, 2012).These result contrast to result of presnt study.

In the present study There was no significant difference between means of Complete blood cell count in pregnant womenwho's age more or less than 30 years.

4.2Conclusion

1- Hct, TRBCs, Hb, MCHC and lymphocytes percentage($p \leq 0.05$) decreased significantly in pregnant women when compared withnon pregnantladies.

2- TWBCs, PDW, MPV and neutrophils percentage increasedsignificantly (P = 0.00) of pregnant ladiesmore thannon pregnant ladies.

3- MCV, MXD% and platelets decreased in pregnantladies(p.value$\geq$ 0.08)when compared withnon pregnantladies.

4- MCH and RDWsd(p.value$\geq$ 0.5) of pregnant ladiesincreasedmore than non pregnantladies.

5-The history of pregnant abortion didn't affect Hb, Hct, TRBCs count and indices and TWBCs count and differential ,platelets and their indices .

6-Thenumber of children didn,t affect Complete blood count in pregnant ladies.

7-The age of pregnant ladies didn,t affect Complete blood count .

4.3Recommendation

- Regular checking of CBC during second trimester of pregnant women

-Regular follow up of pregnant women.

-Pregnant woman should regularly intake supplementation with iron because it affects the hematological parameter of them.

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Appendix

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire to measure CBC of pregnant women in Second` trimester attended in Bahry locality

NO ( )Personal

dataName……Age……Occupation……Husband

occupation……Residence……

Month of pregnancy……NO of

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

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

Other……

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

Results: WBC……RBC……HGB……

HCT……MCV……MCH……

MCHC……PLT……LYM%……

NEUT%……MXD%……LYM#……

NEUT#……MXD……RDW……

PDW……MPV……

Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم ومبحث المناعة الدموية

اقرار موافقه بالمشاركه

الإسم :.....

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

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

أوافق أنا المذكور اعلاه أخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ:.....

Appendix (3)

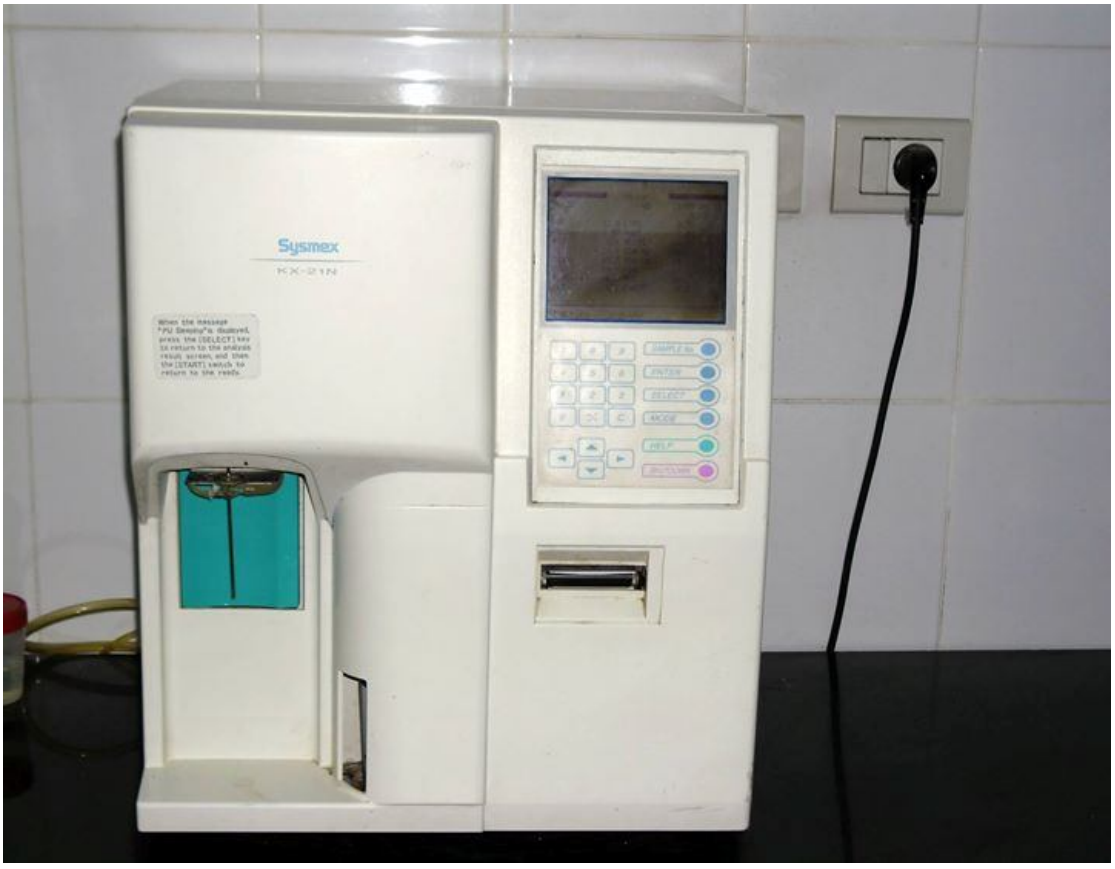

Figure (2.1)Sysmex KX -21N

Red cell distribution width (RDW)

Automated instruments produce volume distribution histograms that allow the presence of more than one population of cells to be identified. Instruments may also assess the percentage of cells falling above and below given MCV thresholds

and ‘flag’ the presence of an increased number of microcytes or macrocytes. The RDW SD is measured by calculating the width in fl at the 20% height level of the red cell size distribution histogram and the RDW CV is calculated mathematically as the coefficient of variation, i.e. RDW(CV)%= 1SD/MCV*100%. The normal reference range is in the order of 12.8±1.2% as CV and 42.5±3.5 fl as SD (Dacie and Lewis, 2011)**..**

Mean corpuscular hemoglobin (MCH)

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

Mean cell hemoglobin concentration (MCHC)

The MCHC is derived in the traditional manner from the Hb and the Hct with instruments that measure the Hct and calculate the MCV. MCHC=Hb / Hct×100. Women normal range 330 ±15 g/L (Dacie and Lewis, 2011).