الآيه

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ وَلَقَدْ خَلَقْنَا ٱلْإِنسَٰنَ مِن سُلَٰلَةٍ مِّن طِينٍ ﴿١٢﴾ ثُمَّ جَعَلْنَٰهُ نُطْفَةً فِى قَرَارٍ مَّكِينٍ ﴿١٣﴾ ثُمَّ خَلَقْنَا ٱلنُّطْفَةَ عَلَقَةً فَخَلَقْنَا ٱلْعَلَقَةَ مُضْغَةً فَخَلَقْنَا ٱلْمُضْغَةَ عِظَٰمًا فَكَسَوْنَا ٱلْعِظَٰمَ لَحْمًا ثُمَّ أَنشَأْنَٰهُ خَلْقًا ءَاخَرَ ۚ فَتَبَارَكَ ٱللَّهُ أَحْسَنُ ٱلْخَٰلِقِينَ ﴿١٤﴾ ﴾

صدق الله العظيم

سوره المؤمنون (الايات 14,13,12)

Dedication

I dedicate this work to source of my happiness my parents thank for giving me a chance to prove and improve myself through all my walks of life and support me to my studies.

Also to my brothers and sister for endless love, support and encouragement.

Acknowledgment

First of all my thanks to ALMIGHTY ALLAA for giving me health strength to accomplished this work

And special thanks to my director and supervisor Dr.Khalda Mirghani Hamza

My thanks also to my brothers and sisters Great thanks to my friends for their help

ABSTRACT

This is a case control study conducted in Khartoum Locality during period from March to October 2015 to determine the complet blood count of Sudanese pregnant women at second trimester..

Eighty healthy pregnant women and forty non pregnant women were informed about the study and agreed for participation. A questionnaire was designed to collect information about the study group such as age, history of abortion, supplement intake and regular clinics visit. 2.5ml of venous blood was take in K_2EDTA anticoagulant container. Automated hematological analyzer (Sysmex KX21N) was used to measure complete blood count.

The results showed that : Significant(0.00) decrease in Hb of pregnant women (11.3±1.4g/dl) compared with non pregnant women (12.1g/dl±1.09). Significant (p=0.00) increase in MCV mean (83.7±8.5 fl), in pregnant women when compared with non pregnant women (76.9 ± 5.6 fl) and significant (P = 0.01) increase in means of neutrophils (5.2±1.8x10^9) of pregnant women more than non pregnant women (4.4±2.5/ L), insignificant increase in mean of TWBCs of pregnant women when compare non pregnant women.

No significant effect on CBC according to, regular clinic visit ,history of abortion , supplemention intake except on MCH significant (P= 0.04) increase in pregnant women had history of aboration and significant (P = 0.02) increase in MCV of pregnant women take supplement when compare with pregnant women not take supplement.

المستخلص

هذه دراسه حاله ضبط تم اجراؤها في الفتره مابين مارس الي اكتوبر 2015 بمحليه الخرطوم لقياس تعداد الدم الكامل في النساء الحوامل خلال الفتره الثانيه من الحمل.

تم اختيار ثمانون عينه من النساء حوامل خلال الفتره الثانيه من الحمل و اربعين من نساءغير حوامل بعد اخطارهن بهذه الدراسه واهميتها واخذت موافقتهن علي المشاركه. صمم استبيان لجمع معلومات عن المشاركات في الدراسه بالنسبه للعمر, تاريخ وجود اجهاض ,المتابعه الطبيه المنتظمه ,واخد المكملات الغذائيه خلال فتره الحمل. تم جمع 2.5مل عينه دم في حاويه تحتوي علي مانع تجلط (ثنائي بوتاسيوم ثنائي امين الايثلين رباعي حمض الخل) من كل مشاركه. واستخدم جهاز(تحليل الدم الاوتوماتيكي) لتحليل تعداد الدم الكامل والدي يعمل اتوماتيكيا.

اظهرت النتائج الي ان تركيز خضاب الدم في الخليه قد انخفض (متوسط 11.3جم/ديسيليتر1.4±) انخفاضا ذا دلاله احصائيه في النساء الحوامل مقارنه بالمجموعه الضابطه (متوسط12.1جم/ديسيليتر1.09±) (مستوي معنويه0.00), وأن متوسط حجم الكريه قد زاد زياده ذات دلاله احصائيه في النساء الحوامل(متوسط 83.7 فيمتولتر8.5±) مقارنه بالمجموعه الضابطه (متوسط 5.6فيمتوليتر76.9±) (مستوي معنويه)0.00 في حين أن الخلايا المتعادله قد زادت بمتوسط (5.210^{9}لتر1.8±) (مستوي معنويه0.01) في النساء الحوامل مقارنه بالمجموعه الضابطه ($4.4\pm$لتر10^{9}/2.5.)

ليست هناك تاثير في تعداد الدم الكامل في النساء الحوامل بالنسبه للزيارات المنتظمه للوحدات الصحيه ,تاريخ وجود اجهاض واخذ المكملات الغذائيه ماعدا تركيز خضاب الدم في الخليه في النساء الحوامل قد زاد زياده ذات دلاله احصائيه (مستوي معنويه = 0.04) الائي لديهن تاريخ بالاجهاض مقارنه بالنساء الحوامل الائي ليس لدين تاريخ بالاجهاض وان هنالك زياد في متوسط حجم الكريه زياده احصائيه (مستوي معنويه = 0.02) في النساء الحوامل الائي ياخذن مكملات غذائيه مقارنه بالحوامل الائي لا ياخذن مكملات غائيه.

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Abbreviations

AGM	Aorta Gonad Mesonephors.
CBC	Complete Blood Count.
DPG	Diphosphoglycerate.
EDTA	Ethylene diamine Tetra Acetic Acid.
HcT	Hematocrit.
LMP	Last Menstrual Period.
MCV	Mean Cell Volume.
MCH	Mean Corpuscular Hemoglobin.
MCHC	Mean Corpuscular Hemoglobin Concentration
PCV	Packed Cell Volume.
RDW	Red cell Distribution Width.
RE	ReticuleEndothelial.
TIBC	Total Iron Binding Capacity.
WBC	White Blood Cell.
WHO	World Health Organization.

CHAPTER ONE

Introduction and literature review

CHAPTER ONE

INTRODUCTION and LITERATURE REVIEW

1.1 Introduction

Pregnancy outcome is influenced by many factors some of which include culture. Environment, socioeconomic status and access to medical care. The hematological profile of pregnant women also has an impact on pregnancy and the outcome of the pregnancy (Ayokunle, 2011).

Pregnancy places extreme stress on the mother body and health, but her systems become prepared for the increased workload and nutritional requirements of the fetus, almost every system is involved so that is a wide range of physiological changes that associated with normal pregnancy (Hoffbrand and Moss, 2011).

In normal pregnancy, there is an increase in erythropotitic activity However, at the same time, an increase in plasma volume occurs, and this results in a progressive decrease in Hb , Hct and RBC The level returns to normal about a week after delivery. There is a slight increase in MCV during the second trimester. Serum ferritin decreases in early pregnancy and usually remains low throughout pregnancy, even when supplementary iron is given (Daice and Lewis, 2011).

The complete blood count (CBC) is one of the most frequently ordered and most time- honored laboratorytests in the hematology laboratory. This evaluation consists of nine components and offers the clinician a variety of hematological data to interpret and review that directly relate to the health of the bone marrow, representedby the numbers and types of cells in the peripheral circulation. The nine components of the CBC are the white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hemoglobin (MCH), mean corpuscularHemoglobin content (MCHC), platelet count, and red cell distribution width (RDW), depending on the type of automated instrumentation used, some of theseParameters are directly read from the instrument and some are calculated (Ciesla,2007).

This study aimed to measure CBC that may be affected during pregnancy. Also the study is included comparison between the measured parameters according to age, ,

using of supplementation, and history of abortion and checkup and visit the clinic regularly in pregnant women in second trimestercomparedto non-pregnant women living Khartoum.

1.2 Literature Review

1.2.1 Haemopoiesis

Hematopoiesis is defined as the production, development, Differentiation, and maturationof all blood cells. Within these four functions is cellular machinery that outstrips most high-scale manufacturers in terms of production quotas, customs specifications, and quality of final product. When one considers that the bone marrow is able to produce 3 billion red cells, 1.5 billion white cells, and 2.5 billion platelets per day per body weight, the enormity of this task in terms of output is, almost incomprehensible (Ciesla,2007).

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta - gonads - mesonephros) region. These common precursors of endothelial and haemopotietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow and from 6 weeks until 6 – 7 months of fetal life the liver and spleen are the Major haemopotietic organs and continue to produce blood cells until about 2 weeks after birth, The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation (Hoffbrand and Moss, 2011).

1.2.1.1 Erythropoiesis

10^{12} new erythrocytesmakes approximately each day by the complex and finely regulated process of erythropoiesis. Erythropoiesis is regulated by the hormone erythropoietin. Erythropoiesis passes from the stem cell through the progenitor cells colony - forming unit granulocyte, erythroid, monocyte and megakaryocyte, burst – forming unit erythroid and erythroid to the first recognizable erythrocyte precursor in the bone marrow, the pronormoblast, Thisis a large cell with dark blue cytoplasm, a centralnucleus with nucleoli and slightly clumpedchromatin. The pronormoblast gives rise to a series of progressively smaller normoblasts by a number of

celldivisions. They also contain progressively more hemoglobin (which stains pink) in thecytoplasm; the cytoplasm stains paler blue as it losesits RNA and protein synthetic apparatus whilenuclear chromatin becomes more condensed The nucleus is finally extruded from the late normoblast within the marrow and a reticulocytestage results which still contains some ribosomalRNA and is still able to synthesize haemoglobin (Hoffbrand and Moss, 2011).

Erythropoiesis is regulated by the hormone erythropoietin. Erythropoietin is a heavily glycosylated polypeptide of 165 amino acids with a molecular weight of 34 kDa. Normally, 90% of the hormone is produced in the per tubular interstitial cells of thekidney and 10% in the liver and elsewhere (Hoffbrand and Moss , 2011).

1.2.1.1.1 Red cell structure and function

The mature red blood cell is a magnificently designed instrument for hemoglobin delivery, anucleate structure with no capacity to synthesize protein, yet it is capable of a limited metabolism, which enables it to survive120 days, An intact, competent, and fully functioning red cell membrane is an essential ingredient to a successful red cell life span. The membrane of the red cell is a trilaminar and three-dimensional structure containing glycolipids and glycoproteins on the outermost layer directly beneath the red cell membrane surface Cholesterol and phospholipids form the central layer, and the inner layer, the cytoskeleton, contains the specific membrane protein, spectrin, and ankyrin(Ciesla, 2007).

1.2.1.1.2 Structure and function of hemoglobin

Hemoglobin is the life-giving substance of every red cell, the oxygen-carrying component of the red cell,consists of two primary structures, Heme portion .This structure involves four iron atoms in the ferrous state (Fe^{2}_ because iron in the ferric state, Fe^{3}_, cannot bind oxygen) surrounded by protoporphyrin IX, or the porphyria ring, a structure formed in the nucleated red cells. Protoporphyrin IX is the final product in the synthesis of the heam molecule. It results from the interaction of succinyl coenzyme A and delta-aminolevulinic acid in the mitochondriaof the nucleated red cells Globin portion. These consist of amino acids linked together to form a polypeptide chain. The most significantchains for adult hemoglobin's are the

alpha and beta chains. Alpha chains have 141 amino acids in a unique arrangement, and beta chains have 146 amino acids in a unique arrangement. The heam and globin portions of the hemoglobin molecule are linked together by chemical bonds, An additional structure that supports the hemoglobin molecule is 2,3-diphosphoglycerate (2,3-DPG), a substance produced via the Embden-Meyerhof pathway during anaerobic glycolysis, This structure is intimately related to oxygen affinity of hemoglobin (Ciesla,2007).

Hematocrit (HcT):

This is some time also referred to a packed cell volume (PCV) or erythrocyte volume fraction .it is the proportion of blood volume that is occupied by red blood cell. For normal subjects it is about 46% for men and 38% for women .hematocrit measurement is considered as integral part of complete blood count result, along hemoglobin concentration, white blood count and platelet count .Most of the modern automated analyzer have the facility to measure hematocrit. Both elevated and depressed value of hematocrit is suggestive of some malfunctioning on the body (Singh, 2010).

Red cell indices:

From the estimated hemoglobin content, HCT, and red cell count, it is possible to drive other value, which indicate the red cell volume .hemoglobin content and concentration in the red cell .these value are commonly referred to as the red cell indices. they are mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean cell hemoglobin concentration (MCHC). The MCV define the volume or size of RBCs, the MCH: define the weight of hemoglobin in the average RBC, the MCHC: define the hemoglobin concentration or color of average RBC(Pal and Pal, 2005).

1.2.1.2 Leucopoiesis

The white blood cells (leucocytes) may be divided into two broad groups: the phagocytes and the immunocytes. Granulocytes, which include three types of cell – neutrophils (polymorphs), eosinophil and basophils – together with monocytes comprise the phagocytes. Only mature phagocytic cellsand lymphocytes are found in

normal peripheralblood. The lymphocytes, their precursor cells and plasma cells, which make up the immunocyte population(Hoffbrand and Moss , 2011).

1.2.1.2.1 Structure and function of leucocytes

Neutrophilsare produced in the bone marrow; they spend 6–10 hours in the blood stream before moving from capillaries into tissues,have a nucleus which stains purple and is divided into two to five segments or lobes. The lobes are separated by a thin strandor 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 by a thin strand or filament of nuclear material. The nuclear chromatin is heterogeneous with some clumping 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 inflammation is known as chemotaxis(Bain, 2004).

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, eosinophil are produced in the bone marrow and circulate in the blood stream for 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 defines against tissue parasites, being able to discharge their granule contents extracellular, seriously damaging large parasites, and also involved in allergic reactions(Bain, 2004).

Basophils**:**have 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 color, 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).

Lymphocytes are produced from lymphoid stem cells in the bone marrow and probably the thymus, Lymphocytes are the second most numerous circulating white

cell after neutrophils. They are smaller than granulocytes with a round or somewhat irregular outline and pale blue, clear cytoplasm. Some lymphocytes have a variable number of azurophilic (pinkish-purple) granules. Lymphocytes are divided into three morphological categories, depending on their size, the amount of cytoplasm and the presence or absence of cytoplasmic granules, These categories are small lymphocyte, large lymphocyte and large granular lymphocyte), Small lymphocytes are most numerous. The nuclear chromatin of lymphocytes may be dense and homogeneous (particularly in small lymphocytes) or more lightly staining and somewhat heterogeneous (particularly in large lymphocytes), 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 humoral immunity. 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. functional categories of lymphocytes are of far more importance than the morphological categories (Bain, 2004).

Monocytesare the largest normal blood cells. They have lobulated nuclei ,cytoplasm which is greyish- blue, is sometimes opaque and may be vacuolated or contain fine azurophilicgranules 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 defenses against bacterial and fungal infections and also ingest and break down dead and dying body cells (Bain, 2004).

1.2.1.3.Thrombopoiesis

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte the megakaryoblast arises by a process of differentiation from the haemopoieticstemcell. The megakaryocyte maturesbyendomitotic synchronous

replication enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two (Hoffbrand and Moss, 2011).

1.2.1.3.1 Structure and function of platelet

Platelets are considerably smaller than red cells and white cells; They are pale blue with fine azurophilic granules which tend to be clustered in the center 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).

1.2.2 Pregnancy

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring develops inside a woman. A multiple pregnancy involves more than one offspring, such as with twins, Pregnancy can occur by sexual intercourse or assisted reproductive technology. It usually lasts around 40 weeks from the last menstrual period (LMP) and ends in childbirth. This is just over nine lunar months, where each month is about 29½ days. When measured from conception it is about 38 weeks. An embryo is the developing offspring during the first eight weeks following conception, after which, the term fetus is used until birth. Symptom of early pregnancy may include a missed periods, tender breasts, nausea and vomiting, hunger, and frequent urination Pregnancy may be confirmed with a pregnancy test(Abman, 2011).

Pregnancy is typically divided into three trimesters. The first trimester: is from week one through 12 and includes conception. Conception is when the sperm fertilizes the egg. The fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the fetus and placenta. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus)(Hopkins,2012).

Minute ventilation is increased by 40% in the first trimester. The womb will grow to the size of a lemon by eight weeks. Many symptoms and discomforts of pregnancy like nausea and tender breasts appear in the first trimester (Campbell and Klocke 2001).

Weeks 13 to 28 of the pregnancy are called the second trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to 20 times its normal size during pregnancy.,although the fetus begins to move and takes a recognizable human shape during the first trimester, it is not until the second trimester that movement of the fetus, often referred to as "quickening", can be felt. This typically happens in the fourth month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before(Stacey *et al*., 2011).

Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready for birth. During the second trimester, the woman's abdomen would have been very upright, whereas in the third trimester it will drop down quite low, and the woman will be able to lift her abdomen up and down. The fetus begins to move regularly, and is felt by the woman. Fetal movement can become quite strong and be disruptive to the woman. The woman's navel will sometimes become convex, "popping" out, due to her expanding abdomen*(* Stacey*et al*., 2011).

1.2.2.1. Physiological changes during pregnancy

Physiological and anatomical change occur during the course of pregnancy to provide suitable environment for growth and development of the fetus ,early change are due to the metabolic demands brought on by the fetus ,placenta and uterus and in part to increasing levels of pregnancy hormone particularly progesterone and estrogen (Collin*et al*., 2013).

Physiological changes divided into basic group: those occurring in the first half of pregnancy and those in the second half. In general ,physiological changes in the first half are consider maternal anabolic changes because they build the capacity of mothers body t0 deliver relatively large quantities of blood ,oxygen and nutrient to the fetus. in the second half of pregnancy .in the second half is a time of maternal

catabolic changes in which energy and nutrient stores, and the heightened capacity to deliver stored energy and nutrient to the fetus , predominate .Approximately 10% of fetal growth is accomplished in the first half of pregnancy ,and remaining 90% occurs in the second half **.**Body water changes: Awomen[s] body gains good deal of water during pregnancy ,primary due to increase volumes of plasma and extracellular fluids ,as well as amniotic fluid. Total body water increase in pregnancy range 7to 10 liters. Abouttwo –third of expansion is intracellular (blood and body tissue)and one third is extracellular (in the space of the cell). Plasma volume begins to increase within a few week after conception and reach a maximum at approximately 34 weeks .Early pregnancy surges in plasma volume appear to be the primary reason that pregnant women feel tired and become exhausted easily .fatigue associated with plasma volume increase in the second and third month of pregnancy decline as compensatory physiological adjustment are made .The increase volume of water in blood is responsible for the dilution effect of pregnancy on blood concentration of some vitamins and minerals (Brown *et al.,*2011)

1.2.2.2. Hematological changes associated with pregnancy

There are both subtle and substantial changes in hematological parameters during pregnancy and the puerperium, orchestrated by changes in the hormonal milieu. A thorough understanding of these is important to avoid both over and under-diagnosing abnormalities. During pregnancy, the total blood volume increases by about 1.5L, mainly to supply the needs of the new vascular bed. Almost 1 liter of blood is contained within the uterus and maternal blood spaces of the placenta, expansion of plasma volume by 25%–80% is one of the most marked changes, reaching its maximum by mid pregnancy. Red cell mass also increases by 10%–20% but the net result is that hemoglobin (Hb) concentration falls. Typically, this is by 1–2 g/dL by the late second trimester and stabilizes thereafter .Women who take iron supplements have less pronounced Hb changes, as they increase their red cell mass proportionately more than those without dietary supplements the increase is approximately 30% over pre-pregnancy values It is hard to define a normal reference range for Hb during pregnancy and the limit for diagnosing anemia. The World

Health Organization has suggested that anemia is present in pregnancy when Hb concentration is, < 11 g/dL Red cell count and hematocrit (Hct) values are likewise lower in pregnancy, white cell count (WBC) is increased in second pregnancy with a typical reference range of 6×10^9–16×10^9 platelet count decreases during pregnancy, particularly in the third trimester this is Termed "gestational thrombocytopenia, (Pavord and Hunt, 2010).

1.2.3. Anemia

Anemia is defined clinically as a blood hemoglobin or hematocrit value that is below the appropriate reference range for that patient, The reference range is derived from the hemoglobin or hematocrit values of a group of persons who are presumed to be without hematologic disease (in other words, normal). It is defined as the range of values containing 95% of the population (two standard deviations above and below the median value) .The reference range needs to be adjusted for the age and sex of the patient since the hemoglobin and hematocrit vary with age and sex (in adults). It should also be adjusted for other factors, such as altitude (the normal range for Denver, Colorado, would be different from that for Death Valley California. However, for general purposes, anemia can be defined as hemoglobin values less than 14 g/dL (140 g/L) in adult men and less than12 g/dL (120 g/L) in adult women (Kern, 2002).

1.2.3.1 Signs and symptoms of anemia

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

koilonychias 'spoon nails' with iron deficiency, jaundice with hemolytic or megaloblastic anaemias (Hoffbrand *et al*., 2006).

1.2.3.2Classification of anemia

The most useful classification is that based on red cell indices and divides the anemia into microcytic, normocytic and macrocytic As well as suggesting the nature of the primary defect, this approach may also indicate an underlying abnormality before overt anemia has developed. In two common physiological situations the mean corpuscular volume (MCV) may be outside the normal adult range. In the newborn for a few weeks the MCV is high but in infancy it is low and rises slowly throughout childhood to the normal adult range. In normal pregnancy there is a slight rise in MCV, even in the absence of other causes of macrocytosis (e.g. folate deficiency). (Hoffbrand *et al*., 2006).

1.2.3.4 Common types of anemia during pregnancy

Most commonly experienced types of anemia during pregnancy are:

1.2.3.4.1 Iron deficiency anemia

Iron deficiency is the most common cause of anemia in every country of the world. It is the most important cause of a microcytic hypochromic anemia, in which the two red cell indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), are reduced and the blood film shows small (microcytic) and pale (hypochromic) red cells. This appearance is caused by a defect in hemoglobin synthesis (Hoffbrand *et al*.,2006).

1.2.3.4.1 .1Distribution of body iron: The concentration of iron in the adult human body is normally about 50 mg/kg in males and 40 mg/kg in females. The largest component is circulating haemoglobin, with 450 mL (1 unit) of whole blood containing about 200 mg of iron Mucth of the remainder is contained in the storage proteins, ferritin and haemosiderin. These are found mainly in the reticuloendothelial (RE) cells of the liver, spleen and bone marrow (which gain iron from breaking down red cells), and in parenchymal liver cells (which normally gain most of their iron from the plasma iron-transporting protein, transferrin (Hoffbrand *et al*., 2005).

1.2.3.4.1 .2. Iron absorption: depends not only on the amount of iron in the diet, but also, and more importantly, on the bioavailability of that iron, as well as the body's needs for iron. Normal diet provides approximately 15 mg of iron daily. Of that iron, digestion within the gut lumen releases about one-half in a soluble form, from which about 3 mg may be taken up by mucosal cells and only about 1 mg (or 5–l0% of dietary iron) transferred to the portal blood in a healthy man. Iron absorption can thus be influenced at several different stages (Hoffbrand *et al* ., 2005)

1.2.3.4.1 .3.Iron metabolism :iron has a pivotal role in many metabolic processes, and the average adult contains 3-5 g of iron, of which two thirds is in the oxygen-carrying molecule haemoglobin. A normal diet provides about 15 mg of iron daily, of which 5-10% is absorbed (1 mg), principally in the duodenum and upper jejunum, where the acidic conditions help the absorption of iron in the ferrous form. Absorption is helped by the presence of other reducing substances, such as hydrochloric acid and ascorbic acid. The body has the capacity to increase its iron absorption in the face of increased demand for example, in pregnancy, lactation, growth spurts, and iron deficiency. Once absorbed from the bowel, iron is transported across the mucosal cell to the blood, where it is carried by the protein transferrin to developing red cells in the bone marrow. Iron stores comprise ferritin, a labile and readily accessible source of iron, and hemosiderin, an insoluble form found predominantly in macrophages. About 1 mg of iron a day is shed from the body in urine, feces, sweat, and cells shed from the skin and gastrointestinal tract. Menstrual losses of an additional 20 mg a month and the increased requirements of pregnancy (500-1000 mg) contribute to the higher incidence of iron deficiency in women of reproductive age (Provan, 2003).

1.2.3.4.1.4.Causes of Iron Deficiency:is due to insufficient iron intake, malabsorption of iron despite adequate intake, or iron loss in excess of iron absorption Inadequate dietary iron may be the cause of iron deficiency during times of greatest iron need, including infancy and early childhood, the adolescent growth spurt, and pregnancy. Inadequate dietary iron may lead to deficient storage iron in menstruating women, particularly those with heavy menstrual bleeding. Multiparous

women are at high risk for iron deficiency: each pregnancy results in the loss of ~500 to 700 mg of iron, and an additional 450 mg is needed to expand the blood volume. On average, 2.5 mg of iron must be absorbed daily over the course of the pregnancy (Kern, 2002).

1.2.3.4.1 .5.The clinical features of iron deficiency anemia: the general symptoms of iron deficiency anemia are those of anemia ofany cause: fatigue, dyspnea on exertion, and dizziness. There are a few signs and symptoms that are relatively unique to iron deficiency anemia, including"spoon" fingernails, glossitis (atrophy of the papillae of the tongue, withburning or soreness), ulcerations or fissures at the corners of the mouth(angular stomatitis), and dysphagia due to esophageal webs or strictures.

The combination of dysphagia, angular stomatitis, and hypochromic anemia has been called the Plummer-Vinson or Paterson-Kelly syndrome.

These extreme signs of iron deficiency are now uncommon. Pica is the habitual consumption of unusual substances. It can be both a manifestation and a cause of iron deficiency. Specific examples of pica include geophagia (consumption of earth or clay), pagophagia (ice), and amylophagia (laundry starch). Food pica is the compulsive eating of one kind of food, often crunchy foods such as celery, potato chips, carrots, or raw potatoes. In most cases, pica is a symptom of iron deficiency and disappears when the iron deficiency is relieved. However, pica can also be a cultural phenomenon and, in these instances, can induce iron deficiency. Laundry starch and clay can impair iron absorption. Laundry starch is also extremely poor in iron, so if starch constitutes a significant proportion of caloric intake, the diet is likely to be deficient in iron (Kern, 2002).

1.2.3.4.1.6 .Laboratories test: The anemia of iron deficiency is classically microcytic (decreased MCV) and hypochromic (increased central pallor in red blood cells . However, in early iron deficiency, the MCV will be normal. Occasional microcytic and hypochromic RBCs may be present on the blood smear (Kern, 2002).

The first step in laboratory evaluation should include serum ferritin or serum iron, TIBC, and iron saturation, If the iron studies indicate iron deficiency, the next step is

to determine the cause, If the iron studies are not consistent with iron deficiency in a patient with a microcytic anemia, the next step is hemoglobin electrophoresis to diagnose _-thalassemia or a hemoglobinopathy. A serum lead level should be done in children whir on deficiency is excluded. A bone marrow examination should seldom be necessary to diagnose iron deficiency, but if the iron studies are indeterminate,
a bone marrow examination should be performed and stained for iron. Marrow deficient in iron usually shows mild erythroid hyperplasia; lateerythroid precursors appear ragged, poorly hemoglobinized (grayish), and small. Storage iron must be completely absent; the presence of any stainable iron in the bone marrow excludes the diagnosis of iron deficiency (Kern, 2002)

1.2.3.4.1 .7.Sequence of events of iron deficiency anemia: Depletion of iron storeswhen the body is in a state of negative iron balance, the first event is depletion of body stores, which are mobilized for hemoglobin production. Iron absorption is increased when stores are reduced, before anemia develops and even when the serum iron level is still normal, although the serum ferritin will have already fallen. (Hoffbrad *et al*., 2005).

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

If the negative balance continues, frank iron deficiency anemia develops. The red cells become obviously microcytic and hypochromic, and poikilocytosis becomes more marked. The MCV and MCH are reduced, and target cells may be present. The reticulocyte count is low for the degree of anemia. The serum TIBC rises and the serum iron falls, so that the percentage saturation of the TIBC is usually less than 10%. The number of erythroblasts containing cytoplasmic iron (Sideroblasts) is

reduced at an early stage in the development of deficiency, and siderotic granules are entirely absent from these cells when iron deficiency anemia is established. The erythroblasts have a ragged, vacuolated cytoplasm and relatively psychotic nuclei. The bone marrow macrophages show a total absence of iron, except where very rapid blood loss outstrips the ability to mobilize the storage iron. Platelets are frequently increased (Hoffbrand *et al*., 2005).

1.2.3.4.2 Megaloblastic anemia

The megaloblastic anemia are a group of disorders characterized by the presence of distinctive morphological appearances of the developing red cells in the bone marrow. The cause is usually deficiency of either cobalamin (vitamin B_{12}) or folate, but megaloblastic anemia may arise because of genetic or acquired abnormalities affecting the function or metabolism of these vitamins or because of defects in DNA Synthesis not related to cobalamin or folate (Hofbrand *et al.***,** 2005).

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

1.2.3.4.2 .1.Clinical features of megaloblastic anemia: many symptoms patients are detected through the finding of a raised mean corpuscular volume (MCV) on a routine blood count. The main clinical features in more severe cases are those of anemia. Anorexia is usually marked and there may be weight loss, diarrhea or constipation. Other particular features include glossitis, angular cheilosis, a mild fever in the more severely anemic patients, jaundice (unconjugated) and reversible melanin skin hyperpigmentation, which may occur with either deficiency. Thrombocytopenia sometimes leads to bruising and this may be aggravated by

vitamin C deficiency in malnourished patients. The anemia and low leucocyte count may predispose to infections, particularly of the respiratory or urinary tracts. Cobalamin deficiency has also been associated with impaired bactericidal function of phagocytes (Hoffbrand *et al.,* 2005).

1.2.3.4.2 .2.Vitamin B_{12} and **folate** both are most causes of megaloplastic anemia, the dietary sources and requirements of vitamin B12 (Cobalamin) is synthesized solely by micro-organisms. Ruminants obtain cobalamin from the foregut but the only source for humans is food of animal origin, The highest amounts are found in liver and kidney (up to 100 μg/100 g) but it is also present in shell fish, organ and muscle meats, fish, chicken and dairy products – eggs, cheese and milk – which contain small amounts (6 μg/L), Vegetables, fruits and all other foods of non-animal origin are free from cobalamin unless they are contaminated by bacteria. Cooking does not usually destroy cobalamin, A normal diet must contains between 5 and 30 μg of cobalamin daily, Adult daily losses (mainly in the urine and faeces) are between 1 and 3 μg (about 0.1% of body stores) and, as the body does not have the ability to degrade cobalamin, daily requirements are also about 1–3 μg. Body stores are of the order of 2–3 mg and are sufficient for 3–4 years if supplies are completely cut off (Hoffbrand *et al.,* 2005).

1.2.3.4.2.3. Folic acid (folat) is actually pteroylglutamic acid, required for the transfer of one-carbon fragments such as methyl groups in numerous chemical reactions , synthesized by higher plants and microorganisms; it is abundant in vegetables, fruit, cereals, and dairy products. Folic acid is heat labile, and much is destroyed by cooking. Therefore, the primary dietary source for folic acid is fresh uncooked fruits and vegetables. It is primarily absorbed in the jejunum. The daily requirement is ~50 _g. The body has stores of approximately 5 to 10 mg, primarily in the liver. An enterohepatic circulation is required for redistribution of hepatic float stores to the rest of the body. Folate stores can be exhausted in a few weeks to a few months—much faster if the enter hepatic circulation is disrupted. Pregnancy is worth special mention here. It is believed that folate deficiency during pregnancy predisposes the fetus to neural tube defects and that folate supplementation will

reduce the risk of such defects. Every woman who is pregnant, or who is considering becoming pregnant, should take folic acid (Kern, 2002).

1.2.3.4.2.4. Causes of folate deficiency: most causes of folate deficiency are due to inadequate diet).Malabsorption is less common, chronic alcoholics are at particular risk for folate deficiency because they are less likely to eat fresh fruits and vegetables and because alcohol interferes with folate metabolism inadequate diet, alcoholism Lack of fresh fruits and vegetables ,malabsorption Gluten-sensitive enteropathy (celiac sprue), tropical sprue , extensive small bowel resection Inflammatory bowel disease (regional enteritis) Rare Causes ,Hemodialysis, Antiepileptic drugs, Antifolate drugs ,increased requirements: chronic hemolytic anemia, psoriasis, pregnancy ,Oral contraceptives (uncommon) ,Exposure to nitrous oxide (NO_2)(Kern, 2002).

1.2.4. Previous study.

A study of haematological profile during normal pregnancy in Chinese women aimed to investigating the haematological profile during pregnancy in Chinese women and comparing these to the established values for white and black women.White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) count were measured on samples of blood obtained during each pregnant trimester and the 12th and 16th week postpartum. During pregnancy, Hb concentration, RBC count, Hct and PLT count were lower. The lower reference values for Hb and PLT count during pregnancy were 95 g/l and 61 x 10^9/l, which were different from those of white and black women. (Shen *et al*.,2010).

Haematological indices in pregnancy applicable across populations: an evaluation in healthy pregnant Jamaican women. The results indicate that the haemoglobin concentration is highest in the first trimester, reaches its lowest point in the second trimester and begins to rise again in the third trimester. The mean haemoglobin concentration was 12.73 ± 1.14 g/dl in the first trimester, 11.41 ± 1.16 g/dl in the second trimester and 11.67 ± 1.18 g/dl in the third trimester, A similar trend in

changes in concentration to that for the hemoglobin mentioned above, is also seen in the packed cell volume and the red blood cell count. In contrast, the mean corpuscular volume and the mean cell hemoglobin had the lowest value in the first trimester, rose to its highest value in the second trimester and then started to decline in the third trimester. The mean corpuscular hemoglobin concentration however, remained fairly constant throughout pregnancy. The white blood cell count changed in a similar way as the mean corpuscular volume and the mean cell hemoglobin. The platelet count decreased from the first trimester to the third trimester(James*et al*.,2008).

Hematologicalprofile of healthy pregnant women in Ibadan, South-western Nigeria: The objective of this study was to provide reference values for Nigerian pregnant women. The study took place at the Adeoyo Maternity Hospital and the University College Hospital, both in Ibadan and the study showed: First trimester: Hemoglobin (Hb), hematocrit (HcT), WBC and platelet counts was decreased to the second trimester and the Third trimester. These results were compared with those of 52 non-pregnant age matched women volunteers as controls whose mean haematological indices. The following haematological indices: WBC, platelet counts, RBC, PCT, and PDW, of women between the trimesters showed statistical significance (p value < 0.001 in each case). The WBC is inversely proportional to the PCT and the MCV in the pregnant women was slightly raised. In this study, pregnancy is characterized by lowest values of haemoglobin parameters in 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*., 2009).

A studyin Port Harcourt Nigeria: Results obtained for the hematological parameters indicate that only Hematocrit (Hct) showed significant differences amongst the three groups; highest amongst subjects in the third trimester and lowest amongst subjects in the second trimester ($p < 0.05$). Hemoglobin concentration (Hbc), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Erythrocyte Sedimentation Rate (ESR) were found to be highest amongst subjects in the second trimester; Red Blood Cell

(RBC) count and White Blood Cell (WBC) count were highest amongst subjects in the first trimester of pregnancy. These differences were however, not statistically significant ($p > 0.05$). Among the anthropometric parameters studied, only weight showed significant differences in the three groups of pregnant subjects ($p < 0.05$); being highest amongst subjects in the third trimester and lowest in subjects in the first trimester(Dapper et al.,2006).

Aassessmentofcomplete blood count of Sudanese pregnant women in Port Sudan city (2012), indicated that WBC of pregnant women with number of pregnancy between (1-3) pregnancies increased insignificantly (p.value> 0.05) compared to those of pregnancy between (4-7) and (7-10). WBC of pregnant women at third trimester increased insignificantly (p.value 0.08) while lymphocytes (mean=25%±7.0) decreased significantly (p.value 0.01) than those women at first and second trimesters Neutrophils of women at third trimester increased significantly compared to those in first and second trimesters ,when compared of different trimesters with control the result was WBCs and neutrophils increased significantly at third trimester (pvalue 0.00) but for lymphocytes was decreased significantly at third trimester (pvalue 0,00),RBC and HcT of pregnant women increased significantly (p.value0.01) with regularly visits to clinics (Khalil, 2012).

At Omdurman Al Saudi Maternity Hospital: Astudy revealed that there were significant decreased in RBCs count, hemoglobin (Hb) and packed cell volume (PCV) of pregnant women compared tonon-pregnant women (P value <0.05) and significant decreased in mean corpuscularvolume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscularhemoglobin concentration (MCHC) of pregnant women (P value <0.05). TWBCscount was increased significantly (P. value< 0.050) in contrast platelets countsignificantly lower than the normal control (P. value <0.05) (Elgari, 2013).

1.3. Rationale

In Sudanese pregnant women many complications may occur andthreatened the mother and fetus lifesuch as anemia of iron or folate deficiency and this research amied to increase knowledge about hematological status of pregnant women to prevent this crises.

Data about complete blood cell count (CBC) of pregnant women are few in Sudan and this research may provide more data which help in future to produce reference range values.

1.4 Objectives

1.4.1. General objective: to determine the complete blood cell count of pregnant women in Khartoum state at second trimester.

1.4.2. Specific objective:

1-To compare the Hb ,HcT ,RBCs, platelet count , MCH, MCHC, WBCs count of pregnant women with non pregnant women.

2-To compare completeblood count of pregnant women with history of abortion with those without histoty of abortion

3-To detect effect of regular clinic visit and supplementation intake on pregnant women at second trimester.

CHAPTER TWO

Materials and Methods

Chapter Two

Materials and Methods

2.1. Study design:

This study is case control study conducted in period from March toOctober2015 to measure complete blood count in pregnant women atsecondtrimester

2.2. Study population:

Study carried out in one hundred and twenty(120) Sudanese women, eighty(80) pregnant women at second and forty(40) non pregnant as control in Khartoum locality.

2.3. Inclusion criteria:

Pregnant women at the second trimester, and all age groups were included.

2.4. Exclusion criteria:

Pregnant women with any diagnostic diseases such as anemia, previous blood transfusion, and typhoid were excluded.

2.5. Data collection:

Data was collected using a questionnaire which was specifically designed to obtain information data that helped in either including or excluding cases

2.6. Sample collection

2.5 ml venous blood was collected from individual under study and dispensed in EDTA container for estimated competed blood count.

2.6.1. Rquriedment of sample collecation:

1- Automated hematological analyzer KX 21N (sysmex) for determination of CBC

2-EDTA

3-Alchol swab 70%alcohol(ethanol)

4-Cotton,Torniquet and Blister

2.6.2. Procedure of sample collecation:

1- Pregnant women either was sat or lid down right on an examination table.

2- the arm was positioned on the armrest so that the vein identified become under some tension and it's mobility was reduced.

3-the skin was cleaned up with 70% alcohol (ethanol) to dry.

4- Personal detailed were check up on the form and on blood vials

5-Atourniquet was applied to the arm ,tight sufficiently to distened the vein but not cause discomfort.

6- 2, 5 ML of blood sample were taken from the superficial vein of the forearm.

7- Blood was collected in K_2 EDTA anticoagulant container

8- EDTA,blood sample were analyzed by sysmex 21(Dacie and Lewis,2006).

2.7. Procedure of complete blood count (CBC)

Fully automated multichannel instruments(sysmex21) require only that an appropriate blood sample is presented to the analyzer 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).

2.7.1. Principle of sysmex model 21 hematological analyzer:

Measurement of blood cells (RBCs ,WBCs ,and platelet count). And hemoglobin concentration obtained by aspiration of small volume of well mixed (EDTA) blood by sample probe and mixed with isotonic diluent in nublazer. Diluted aspiration delivered to RBCs asperture bath for providing information about RBCs and platlet. Other portion of aspiration sample induced into WBCs bath in which hemolytic reagent (stromatolyzer) added to break dowon (RBCs) and realese of hemoglobin which measured in build colorimeter. Based in cyanomethmoglobin method (HICN).The through three sensing apertures for each cell type, cell counted and size information generated in triplicate pulses acting to electronic conductively. mentioned pluses convert into digital number using in build calculator programmed and designed for RBCs WBCs ,count .some portion of diluted sample delivered to in build hemoglobin meter at the same time. Hence three value directly measured (RBCs,Hb) and displayed on (LCD) other value of red cell indices ,leukocyte

differential and absolute count calculated from given information ,the result printed out according to the setting mode .on the other hand platlet count and histogram determined from pulses acting to size of platelet (Dacie and Lewis,2006).

2.7.2. Hemoglobin concentration (HGB or HB)

Automated counter used nonhazardous chemical, such as sodium lauryl sulphate, imidazole, and sodium dodecyl sulphate or dimethyl laurylamineoxide. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A detergent is included to ensure rapid cell lysis and to reduce turbidity. Measurements of absorbance are made for hemoglobin measurement at various wavelengths depending on the final stable haemochromogen, cyanmethaemoglobin, oxyhaemoglobin, methaemoglobin or monohydroxyferri-porphyrin. Hemoglobin concentration values, in normal female 135 ± 15 g/L, in pregnancy1st trimester 124–135 g/l, 2nd trimester 110–117 g/l, and 3rd trimester 106–109 g/l, 120 g/l or higher may be found when supplementary iron is being given (Dacie and Lewis, 2011).

2.7.3. 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).

2.7.4. Packed cell volume (PCV)

The haematocrit or packed red cell volume (PCV) refers to the proportion of the volume of red cells relative to the total volume of the blood.

Automated blood cell counter was estimated PCV/haematocrit by technology that has little connection with packing red cells by centrifugation. The passage of a cell through the aperture of an impedance counter leads to the generation of an electrical pulse, the of which is proportional to cell volume. The number of pulses generated

allows the RBC to be determined. Women normal range 0.41 ± 0.05 L/L (Dacie and Lewis, 2011).

2.7.5. Red cell indices

2.7.5.1. Mean cell volume (MCV)

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

2.7.5.2. 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).

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

2.7.6. Total white blood cell count (WBC)

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

2.7.6.1. Automated differential count

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

neutrophils 40- 80 %, lymphocytes 20-40 %, monocytes 2-10 %, eosinophils 1-6 %, basophils < 1-2% (Dacie and Lewis, 2011).

2.8. Data analysis:

Data interd in computer and analyzed by SPSS(software program) to obtain stander deviation and P value by independent sample T test.

2.9. Ethical consideration:

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

CHAPTER THREE

RESULTS

Chapter three

3.1.Results

This study was carried out at KhartoumLocality during the period from March to October 2015 to determine Hematological parameter of pregnant women at second trimester. Eighty (80)pregnant women(ages between 18-41years) and forty(40) non pregnant women were enrolled. Theresults showed significant decrease in Hb in pregnant women when compared with control group.Significant increase in of MCV of pregnant women compared with control ,and insignificant increased in of HcT in pregnant women when compare with control group, no significant different in mean of TRBCs ,MCH, MCHCbetween pregnant and control groups (table 3.1) .

Significant increased in neutrophils in pregnant women compared with control group.No significancet difference in TWBCs count and lymphocytes, insignificant decreased in platelet count in pregnant women observed compared with control group(table 3.2).

Significantly increased in MCH in pregnant womenwith history of abortion when compared withno history of abortion. Insignificant increase in the mean HCT in positive history of abortion, insignificant deference in HcT , Hb, TRBCs, MCV, MCHC between positive and negative history of abortion (table3.3)

Insignificant different between mean of TWBCs, neutrophils, lymphocytes and platelet count of positive and negative history of abortion, (table3.4).

There was significant increased in mean of MCV of pregnant women take supplement when compared with those no taking supplement pregnant women. There was insignificant increased in the mean of HCT, Hb, pregnant women using supplements when compare with those non using supplement.No Significant different in mean of TRBCs, MCH, MCHC, in using supplement pregnant women compare with those did not take supplement (table 3.5)

Significantdecreased in the mean of TWBCs in using supplement pregnant women compared with non-using group. No significant different in mean of lymphocytes, neutrophils, and platelet in supplement using pregnant women when compared with non-using pregnant women (table3.6).

According to follow up practice of pregnant women ,no significant difference was observed in CBC compared to non-pregnant women (table 3.7, 3.8).

Table (3.1) Comparison between pregnant and non pregnant women with regard to Hb, HCT, RBCs count and RBCs indices

Test	Sample	No	Mean±SD	P value
Hbg/dl	Pregnant	80	11.3±1.4	0.00
	Non pregnant	40	12.1±1.09	
HCT %	Pregnant	80	34.3±3.4	0.07
	Non pregnant	40	33.2±2.7	
TRBCs $\times 10^{12}$ /l	Pregnant	80	4.1±0.4	0.07
	Non pregnant	40	4.3±0.6	
MCV FL	Pregnant	80	83.7±8.5	0.00
	Non pregnant	40	76.9±5.6	
MCH pg	Pregnant	80	27.8±3.3	0.3
	Non pregnant	40	27.4±2.3	
MCHC %	Pregnant	80	32.6±2.1	0.06
	Non pregnant	40	35.8±1.4	

Table (3.2) Comparison between pregnant and non pregnant women with regard to TWBCs count, differential and platelet count.

Test	Sample	No	Mean ±SD	P value
TWBCs $\times 10^9$ /l	Pregnant	80	7.8±2.2	0.2
	Non pregnant	40	7.2±2.6	
Lymphocyte $x10^9$	Pregnant	80	2.4±0.9	0.3
	Non pregnant	40	2.2±0.6	
Neutrophil $x10^9$ /L	Pregnant	80	5.2±1.8	0.01
	Non pregnant	40	4.4±2.5	
Platelet $x10^9$ /L	Pregnant	80	255±62	0.1
	Non pregnant	40	276±75.3	

Table (3.3) effect of history abortion on Hb, HcT, RBCs count and indices of pregnant women at second trimester.

Test	History of abortion	No	Mean ±SD	P value
Hb g/dl	yes	32	11.4±1.7	0.6
	no	48	11.2±1.2	
HCT %	yes	32	35±2.8	0.06
	no	48	33±3.6	
TRBCs $\times 10^{12}$ /l	yes	32	4.1±0.5	0.9
	no	48	4.1±0.4	
MCV FL	yes	32	83.3±10	0.7
	no	48	84±7.4	
MCH pg	yes	32	28.5±5	0.04
	no	48	27.4±2.4	
MCHC %	yes	32	32.9±1.5	0.3
	no	48	32.4±2.4	

Table (3.4) effect of history abortion on TWBCs count, differential and plateletcount of pregnant women at second trimester.

Test	History of abortion	NO.	Mean±SD	P value
TWBCs $\times 10^9$ /l	yes	32	8± 1. 8	0.4
	no	48	8± 2.3	
Lymphocytes $\times 10^9$ /l	yes	32	2.2±1.3	0.2
	no	48	1.9±0.4	
Neutrophils $\times 10^9$ /l	yes	32	4.9±1.5	0.1
	no	48	5.5±1.9	
Platelet x10^9\l	yes	32	266±57	0.2
	no	48	248±62	

Table (3.5) Supplemation intake effect on Hb, HcT, MCV, MCH,MCHC, and TRBCS countof pregnant women at second trimester.

Test	Supplemention intake	No	Mean±SD	P value
Hb g/dl	yes	69	11.3±1.5	0.4
	no	11	10.9±0.9	
Hct %	yes	69	34.6±3.4	0.06
	no	11	32.6±2.2	
TRBCs $\times 10^{12}$ /l	yes	69	4.1±0.45	0.7
	no	11	4.1±0.34	
MCV fl	yes	69	82.9±8.7	0.02
	no	11	79±4.5	
MCH pg	yes	69	27.7±2.3	0.2
	no	11	28±1.8	
MCHC %	yes	69	32.8±1.9	0.08
	no	11	32.6±1.8	

Table (3.6) Supplemation intake effect on TWBCs count, differential and plateletcount of pregnant women at second trimester.

Test	Supplemention intake	No	Mean±SD	P value
TWBCs $\times 10^9$ /l	yes	69	7.5±1.9	0.01
	no	11	9.3±3	
Lymphocytes $\times 10^9$ /l	yes	69	2.1±0.9	0.2
	no	11	2±0.5	
Neutrophils $\times 10^9$ /l	yes	69	5.1±1.7	0.09
	no	11	6.1±2.3	
Platelet $\times 10^9$/l	yes	69	252±257	0.3
	no	11	274±274	

Table (3.7) Regular clinics visit effect on Hb, TWBCs count and indices and HCT of pregnant women at second trimester.

Test	Clinical follow up	No	Mean±SD	P value
Hb g/dl	yes	43	11.3±1.3	0.07
	no	37	10.8±1.5	
Hct %	yes	43	34.4±3.8	0.5
	no	37	33.9±2.9	
TRBCs $\times 10^{12}$ /l	yes	43	4.2±0.4	0.3
	no	37	4.1±0.3	
MCV fl	yes	43	81±9.5	0.8
	no	37	81.8±7.1	
MCH pg.	yes	43	27.4±2.5	0.5
	no	37	27±2.4	
MCHC %	yes	43	32.4±2	0.2
	no	37	31.9±2.7	

Table (3.8) Regular clinics visit effect on TWBCs count,differential and platelet countof pregnant womenat second trimester.

Test	Clinical follow up	No	Mean±SD	P value
TWBCs $\times 10^9$ /l	yes	43	7.7±2.0	0.3
	no	37	8±2.3	
Lymphocytes $\times 10^9$ /l	yes	43	2.0±0.4	0.4
	no	37	1.8±0.6	
Neutrophils $\times 10^9$ /l	yes	43	5.1±1.6	0.4
	no	37	5.4±2	
	no	37	6.0±1.4	
platelet$x10^9$	yes	43	254±56	0.8
	no	37	257±66	

CHAPTER FOUR

Discussion, Conclusion, and Recommendation

CHAPTER FOUR

Discussion, Conclusion, and Recommendation

4.1. Discussion

This is study was carried out at Khartoumstate during the period from March toOctober 2015 todetermine the hematological parameter of pregnant women at second trimester.

-There was significant decrease in Hb in pregnant women when compared with control(P value =0.00), similar finding was reported in Sudan by Khalil (2012) and Elgari (2013). They indicated that Hb of pregnant women significantly decrease compared to non pregnant women. Also agreed with a study conducted in China by Shen (2010), and in Jamaican by James (2008).Who found the hemoglobin concentration declines from first trimester reachesthe lowest concentration in second trimesterand rise in third trimester, the mean was 12.73 ± 1.14 g/dl in the first trimester, 11.41 ± 1.16 g/dl in the second trimester and 11.67 ± 1.18 g/dl in the third trimester.

The main cause of Hb reducation due to increases Blood plasma volume by approximately 1250 mL, or 45%, above normal by the end of gestation and although the red cell mass itself increases by some 25% this still leads to a fall in Hb concentration (Hoffbrand and Moss, 2011).

Agreater expansion of plasma volume relative to increase in hemoglobin mass and erythrocyte volume is responsible for the modest fall in hemoglobin level observed in healthy pregnant women (Khalil, 2012)

Other studies showed that the increased in plasma volume is associated with normal pregnancy causing diluation of many circulating factor , particulary is the RBCs although pregnancy is associated with an increase in the production of erythrocytes this increase is out stripped by the relative increase in plasma volume (Bakar, 2006).

There was significant increased in mean of MCV (p=0.00) ,similar result obtain

injamicadone by James(2008) indicated that the mean corpuscular volume had the lowest value in the first trimester, rose to its highest value in the second trimester and then started to decline in the third trimester.

Red cell count and hematocrit (Hct) values are likewise lower in pregnancy, but the other red cell indices change little ,although red cells show more variation in size and shape than in the non-pregnant state. There is a small increase in mean cell volume (MCV), of on average 4 fL for iron-replete women, which reaches a maximum at 30–35 weeks gestation and occurs independently of any deficiency of B_{12} and Folate (Pavord and Hunt,2010).

There was significant increase in mean of neutrophils in pregnant women was observed compared with control group (p= 0.01), similar findings were reported by Khalil(2012), which showed significant increase in neutrophilsin pregnant women compared with control(P value 0.0).

The increase in circulating segmentd neutrophils and granulocytes whose absolute number is ready doubled at term, the reason for the increased leukocytosis is unclear , but may be caused by elevated estrogen and cortisol levels (Gabbe, et al ., 2012).

The results of this study showed insignificant decrease in mean of platelet in pregnant women when compared with control grou(p=0.1) , which with agreed with astudy in Nigiria and China by Akingbola (2006) , Shen (2010) respectively.

Since normal pregnancy is characterized by an increase platelets aggregation and adecrease in number of circulating platelet with gestation , increase consumption of platelet in utroplacental circulation has been suggested to be the explanation of the reduction in the number of circulating platelets (Juan *et al .,*2011).

There was asignificant increase in the mean of MCH in pregnant women whom had history of abortion when compared with those with no history of abortion (p=0.04), these finding disagreed with the result by Khalil (2012) who indicated that no singnificant different between positive and negative history of abortion.

Insignificant increase in the mean of platelet and HCT in positive history of abortion (p=0.06), which agreed with a study by Khalil (2012) mean of HcT for positive history (35± 4.2 %), and for negative history (34.0±3.1%).

No significant different in Hb, MCV, TRBCs and MCHC($p \geq 0.9$) ,and also no significant different TWBCs, lymphocytes , and neutrophils in pregnant women had history of abortion when compare with pregnant women have negative history with abortion($p \geq 0.1$),insignificant increase in mean of platelet in positive history of abortion(P=0.2).

There was significant increase in mean of MCV of using supplements pregnant women when compare with non-using supplement (p=0.02), this may due to deficient to folic acid which lead to microcytic cell and low MCV.

Significant decrease in mean of TWBCs count in using supplement pregnant women compared with not-using supplement (p=0.01).

According to regular follow up no significant difference in hematological parameter studed.

4.2.Conclusions

1-There was a significant decrease in mean of Hb and there was significant increasein the MCV and neutrophilsin pregnant women at second trimester when compared with non pregnant women.

2-There was a significant increase in the mean of MCH in pregnant women whom had history of abortion when compared with those with no history of abortion.

3-There was a significant increasein MCV of taking supplements pregnant women when compare with not taking supplement, and significant decrease in mean of TWBCs count inpregnant women using supplement.

4.3 Recommendations.

1-In Sudan as developing country, pregnancy complications are expected and to minimize these complications, so pregnant women showed receive more special medical care.

2- Hb , MCV, TWBCs and platelet showed be determined regularly at second trimester.

3- In Sudan ,data concerning hematological profile of pregnant women are few so future studies with large sample size are needed to help health authorities to implement polices to improve health status of pregnant women .

4- Determine normal reference value to Sudanese pregnant women.

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Appendix

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

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

NO ( )Personal dataName……..Age…… ……. Month of pregnancy……Abortion: yes ( ) how many times ( ) No ( )

Supplementation intakeyes ( ) No ( )

Regular( ) Irregular ( )

Visit to clinic yes ( ) No ( )

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

Other…… Results:

WBC….....

RBC…… HGB……...…….

HCT……..... MCV……...

MCH……..……. MCHC……..…

PLT…….

LYM#..... NEUT#.....

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 (2.5 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)

Principle of sysmex model21 hematological analyzer:

Measurement of blood cells (RBCs ,WBCs ,and platelet count). And hemoglobin concentration obtained by aspiration of small volume of well mixed (EDTA) blood by sample probe and mixed with isotonic diluent in nublazer. Diluted aspiration delivered to RBCs asperture bath for providing information about RBCs and platlet. Other portion of aspiration sample induced into WBCs bath in which hemolytic reagent (stromatolyzer) added to break dowon (RBCs) and realese of hemoglobin which measured in build colorimeter. Based in cyanomethmoglobin method (HICN).The through three sensing apertures for each cell type ,cell counted and size information generated in triplicate pulses acting to electronic conductively.mentioned

pluses convert into digital number using in build calculator programmed and designed for RBCs WBCs ,count .some portion of diluted sample delivered to in build hemoglobin meter at the same time. Hence three value directly measured (RBCs,Hb) and displayed on (LCD) other value of red cell indices ,leukocyte differential and absolute count calculated from given information ,the result printed out according to the setting mode .on the other hand platlet count and histogram determined from pulses acting to size of platelet (Dacie and Lewis,2006).