Sudan University of Science and Technology
College of Graduate Studies

Determination of Complete Blood Cell Count of Sudanese Pregnant Women at the Third Trimester attended Khartoum Teaching Hospitals

تحديد تعداد الدم الكامل عند النساء الحوامل خلال الفتره الثالثة من الحمل بمستشفيات الخرطوم التعليمية

A dissertation Submitted in Partial Fulfillment of the Requirement for M.Sc. Degree in Medical Laboratory Science
(Hematology and Immunohematology)

Submitted by:
Khawla Mohammed Nasor Awadelkareem
B. Sc. Shendi University (2008)

Supervisor:
Dr. Khalda Mirghani Hamza

December 2015

الآية

بسم الله الرحمن الرحيم

﴿ وَوَصَّيْنَا ٱلْإِنسَٰنَ بِوَٰلِدَيْهِ إِحْسَٰنًا ۖ حَمَلَتْهُ أُمُّهُۥ كُرْهًا وَوَضَعَتْهُ كُرْهًا ۖ وَحَمْلُهُۥ وَفِصَٰلُهُۥ ثَلَٰثُونَ شَهْرًا ۚ حَتَّىٰٓ إِذَا بَلَغَ أَشُدَّهُۥ وَبَلَغَ أَرْبَعِينَ سَنَةً قَالَ رَبِّ أَوْزِعْنِىٓ أَنْ أَشْكُرَ نِعْمَتَكَ ٱلَّتِىٓ أَنْعَمْتَ عَلَىَّ وَعَلَىٰ وَٰلِدَىَّ وَأَنْ أَعْمَلَ صَٰلِحًا تَرْضَىٰهُ وَأَصْلِحْ لِى فِى ذُرِّيَّتِىٓ ۖ إِنِّى تُبْتُ إِلَيْكَ وَإِنِّى مِنَ ٱلْمُسْلِمِينَ ﴿١٥﴾ ﴾

صدق الله العظيم

سورة الأحقاف (الآية *15*)

Dedication

To my grandfather

To my father

To my mother

*To my brother*s

To my sisters and Selma

To my uncles and aunts

Acknowledgments

Firstly I would like to thanks God for help. And grateful to my supervisor Dr. Khalda Mirghani for her advice and encouragement to conduct this study. My thanks also to my friends for their strong assistance while conducting my study.

Abstract

This is a case control study conducted at Khartoum state during the period from April to October 2015. The aim of this study is to determine the complete blood cell count of Sudanese pregnant women at third trimester of pregnancy.

Eighty healthy pregnant women and 40 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, number of pregnancies and history of abortion in pregnant women. Three ml of venous blood were taken in K_2 EDTA anticoagulant container. Automated hematological analyzer (Mindary BC-3000 plus) was used to measure Complete Blood Count.

The results showed that RBCs, Hb, MCHC, lymphocyte count, lymphocyte% decreased significantly in pregnant women (4.0×10^6/µl ± 0.46), (10.9g/dl ±1.24), (32.4g/dl±1.72), (1.9×10^3/µl± 0.59), (26.7% ±8.02) and (223×10^3/µl±61.0) compared to non pregnant women (4.3×10^6/µl ± 0.38), (12.2g/dl ±1.07), (36.1/dl± 2.07), (2.3×10^3/µl± 0.66), (37.9% ± 13.85) and (292×10^3/µl± 74.88) respectively (P.value = 0.00). While MCV, neutrophil count and neutrophil% increased significantly in pregnant women (84.6fl ±5.91), (4.9×10^3/µl±1.49) and (66.0% ±8.05) compared to non pregnant women (78.5fl ± 6.81), (4.1×10^3/µl± 2.59) and (55.0% ±16.74) (P.value ≤ 0.04). No significant differences in HCT, MCH, WBCs, and MXD of pregnant women when compare to non pregnant women (P.value ≥ 0.08).

No significant effect of abortion, number of children and age on CBC of pregnant women.

In conclusion, pregnancy affect RBC, Hb, MCV, MCHC, neutrophil, and lymphocyte significantly.

المستخلص

هذه دراسة حالة ضبط تم إجراؤها في الفترة ما بين أبريل إلى أكتوبر 2015 بمدينة الخرطوم لتحديد تعداد الدم الكامل للنساء الحوامل في الثلث الثالث من الحمل في مستشفيات الخرطوم .

أخذت ثمانون عينة من نساء حوامل أصحاء و اربعون عينة من غير الحوامل بعد إخطارهن بهذه الدراسة وأخذت موافقتهن على المشاركة. صمم إستبيان لجمع معلومات عن المشاركات في الدراسة بالنسبة للعمر، عدد مرات الحمل و تاريخ حدوث إجهاض للنساء الحوامل. تم جمع عينة دم 3 مل في حاوية تحتوي على مانع تجلط (ثنائي أمين الإيثلين رباعي حمض الخل) من كل مشاركة. تم إستخدام جهاز مندري لتحليل تعداد الدم الكامل.

أشارت النتائج إلى أن كريات الحمراء, خضاب الدم, متوسط تركيز خضاب الدم في الخلية, عدد الخلايا اللمفاوية, نسبة الخلايا اللمفاوية و الصفيحات الدموية قد إنخفض إنخفاضا ذو دلالة احصائية في النساء الحوامل (4.0×10^6/مايكروليتر±0.46), (10.9جم/ديسليتر±1.24), (32.4جم/ديسليتر± 1.72), (1.9×10^3/مايكروليتر ±0.59), (26.7× 10^3/مايكروليتر ±8.02) و(223×10^3/ مايكروليتر±61.0) مقارنة بالمجموعة الضابطة (4.3 × 10^6/مايكروليتر±0.38), (12.2جم/ديسليتر ±1.07), (36.1جم/ديسليتر±2.07), (2.3×10^3/مايكروليتر ±0.66), (37.9× 10^3/مايكروليتر ±13.85) و (292×10^3/ مايكروليتر±74.88) على التوالي بمستوى معنوية (0.00) في حين أن متوسط حجم الكرية الحمراء, عدد الخلايا العدلى و نسبة الخلايا العدلى قد ازداد زيادة ذات دلالة إحصائية في الحوامل (84.6 فيمتوليتر±5.91), (4.9×10^3 /مايكروليتر±1.49) و (66.0%±8.05) مقارنة بالمجموعة الضابطة (78.5فيمتوليتر±6.81), (4.1×10^3 /مايكروليتر±2.59) و (55.0%±16.74) على التوالي بنسبة معنوية(≤0.04) . لا يوجد اختلاف ذو دلالة إحصائية في الهيماتوكريت, متوسط خضاب الخلية, الكريات البيضاء و الخلايا متوسطة الحجم في النساء الحوامل مقارنة بالمجموعة الضابطة(مستوى معنوية ≤0.08).

لا تأثير إحصائي للإجهاض, عدد الأطفال و العمر على تعداد الدم الكامل بالنسبة للنساء الحوامل.

خلصت الدراسة أن الحمل يؤثر على كريات الحمراء, خضاب الدم, متوسط تركيز خضاب الدم في الخلية, عدد الخلايا اللمفاوية, نسبة الخلايا اللمفاوية , الصفيحات الدموية, متوسط حجم الكرية الحمراء, عدد الخلايا العدلى و نسبة الخلايا العدلى.

Table of Contents

List of tables

List of abbreviations

CBC: complete blood count

EDTA: ethylene diamine tetra acetic acid

Hb: Hemoglobin

HCT: Hematocrit

IDA: Iron deficiency anemia

LNMP: Last Normal Menstrual Period

MCH: Mean cell hemoglobin

MCHC: Mean cell hemoglobin concentration

MCV: Mean cell volume

MXD: Mixed (Monocyte- Eosinophil- Basophil)

NTDs: Neural Tube Defects

PLT: Platelet

RBCs: Red blood cell count

RNA: Ribo nucleic acid

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION and LITERATURE REVIEW

1.1 Introduction

Pregnancy places extreme stresses on the haematological system and an understanding of the physiological changes that result is obligatory in order to interpret any need for therapeutic intervention (Hoffbrand and Moss, 2011).

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).

Hematological profile is measured all over the world to estimate general health, because it is a reliable indicator and is a simple, fast and cost-effective test. In addition, the hematological profile is considered to be one of the factors affecting pregnancy and its outcome. During pregnancy, changes occur and can be observed in hematological indices such as red blood cell (RBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Some of these are decreased – for example, RBC and PLT counts – partly as a result of the physiological hemodilution that occurs in pregnancy, while others are increased, such as the WBC count (Akinbami, *et al.*2013).

Examination of hematologic parameters often yields important diagnostic information and allows broad differential diagnostic impressions to be formed, directing further, more specific testing (Greer *et al.*2003).

The most significant hematological changes are physiologic anemia, neutrophilia, mild thrombocytopenia, increased procoagulant factors, and diminished fibrinolysis (Paidas, *et al.*2012).

This study aimed to compare the haematological parameters of pregnant women at the third trimester with non-pregnant women.

1.2 Literature Review

1.2.1 Haemopoiesis

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

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoiesis 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 haemopoietic 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 haemopoietic organs and continue to produce blood cells until about 2 weeks after birth, The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses; 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

Approximately 10^{12} new erythrocytes are produced 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. This is a large cell with dark blue cytoplasm, a

central nucleus with nucleoli and slightly clumped chromatin. The pronormoblast gives rise to a series of progressively smaller normoblasts by a number of cell divisions. They also contain progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm stains paler blue as it loses its RNA and protein synthetic apparatus while nuclear chromatin becomes more condensed the nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte stage results which still contains some ribosomal RNA 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 hormones produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere (Hoffbrand and Moss, 2011).

1.2.1.1.1 Red blood cells

Red blood cells are simple cells that perform an essential function: the exchange of respiratory gasses. These cells are faced with a number of challenges. They lack nuclei, ribosomes, and other cellular organelles and have no protein synthesis or repair capability. They carry oxygen, a toxic substance, but cannot use it in the process of energy generation like other cells of the body. They must maintain a high internal concentration of potassium and low internal concentration of sodium, against the concentration gradient for both ions, which requires energy. They must be flexible to squeeze through small capillaries and must also with stand the shear stresses of blood flow at high arterial pressures. Erythrocytes are nucleated cells containing few organelles; a large proportion of their cytoplasm consists of the iron containing oxygen transport molecule hemoglobin. Erythrocytes are shaped like biconcave disks approximately 7 to 8 _m in diameter. The biconcave disk shape gives red blood cells (RBCs) the flexibility to squeeze their way through capillaries and other small blood vessels. Erythrocytes are the most common cells in blood. The normal RBCs count is approximately 4.5 to 6 million cells per microliter (Kern, 2002).

Red blood cell counts are commonly performed hematology test that are usually part of the complete blood count. The RBC count approximates the number of circulating red blood cell and is helpful in diagnosing and treating of many diseases, especially anemias (Estridge, *et al.* 2000).

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. In 4 months, or120 days, red cells with normal hemoglobin content submit to the rigors of circulation. There are three types of hemoglobin that are synthesized embryonic hemoglobins: fetal hemoglobin, and the adult hemoglobins. Oxygen delivery is the principal purpose of the hemoglobin molecule. Additionally, it is a structure capable of pulling CO_2 away from the tissues, as well as keeping the blood in a balanced pH (Ciesla, 2007).

Haemoglobin is the iron-containing protein found in the red blood cells that transports oxygen to the tissues. Hemoglobin forms an unstable, reversible bond with oxygen. in the oxygenated state, it is called oxyhemoglobin and it is bright red, and in the reduced state, it is purplish blue and may be referred to as deoxy-hemoglobin(Rogers, 2011).

1.2.1.1.3 Hematocrit and red cell indices

Literally, hematocrit means 'blood separation'. The hematocrit measure the percentage of volume of the packed red cells. Hematocrit is a reliable index of the red cell population in the blood. The red cell indices include the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC). The MCV is important in the evaluation of erythrocyte disorders. The MCH and MCHC are generally not of great value (Kern, 2002).

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), eosinophils and basophils together with monocytes

comprise the phagocytes. Only mature phagocytic cells and lymphocytes are found in normal peripheral blood the lymphocytes, their precursor cells and plasma cells, which make up the immunocytes population (Hoffbrand and Moss, 2011).

White blood cells are called leukocytes; their primary function in the body is to defense the tissue against infection and substances foreign to the body. A normal adult has approximately 7000 white blood cells per cubic millimeter of blood (Khalil, 2012).

Leukocytes are usually divided into granulocytes, which have specific granules, and agranulocytes, which lack specific granules. Granulocytes are divided into neutrophils, eosinophils and basophils. Agranulocytes are divided into lymphocytes and monocytes. Although they are called white blood cells, leukocytes predominantly function in tissues, they are only in the blood transiently, while they travel to their site of action (Kern, 2002).

1.2.1.2.1 Neutrophil

Neutrophils are the most common type of WBCs in adults. Two types are described: segmented neutrophils and banded neutrophils. The primary function of neutrophils is phagocytosis, predominantly of bacteria; neutrophils are the primary defense against bacterial infection. Bacteria are killed by antimicrobial agents contained or generated within neutrophil granules. Neutrophils circulate in the blood for ~10 hours and may live 1 to 4days in the extra vascular space. The trip is one way; once neutrophils leave the blood to enter tissues, they cannot return. A significant number of neutrophils are rolling along the endothelial surface of blood vessels (the marginating pool). This population can be rapidly mobilized with acute stressor infection (Kern, 2002).

1.2.1.2.2 Eosinophil

Eosinophils contain large granules that stain reddish-orange (eosinophilic)with usual blood smear stains, the nucleus is segmented (often bilobed).Functions of eosinophils include phagocytosis of antigen-antibody complexes and defense against parasitic

infection. The normal eosinophil count is ~2 to 4% of total WBC. The number of eosinophils increases with allergic reactions and parasitic infections (Kern,2002).

1.2.1.2.3 Basophil

Basophils contain large dark blue or purple (basophilic) granules, which often obscure the nucleus. The nucleus is segmented. Basophils are the least common type of leukocytes, normally ≤1% of total WBCs. The basophil granules contain heparin (an anticoagulant), histamine (a fast vasodilator), the slow-reacting substance of anaphylaxis (a slow vasodilator), and other compounds. Basophils appear to be involved in immediate hypersensitivity reactions related to immunoglobulin class E (IgE) (Kern, 2002).

1.2.1.2.4 Lymphocyte

Lymphocytes are the second most common type of leukocytes in adults (~20–40% of WBC). The lymphocyte number is higher in children and also increases with viral infections. Lymphocyte occurs in two types: B-lymphocyte and T- lymphocyte. B-lymphocyte: cells are the primary effectors of the humoral (antibody-mediated) immune system, they develop in the bone marrow and are found in lymph nodes, the spleen and other organs, as well as the blood. After antigen stimulation, B cells may develop into plasma cells, which are the primary antibody-producing cells. T-lymphocyte are the main effectors of cell-mediated immunity, are the command and control cells of the entire immune system, they stimulate or inhibit the function of other cells of the immune system, including B cells, monocytes and macrophages, and other T cells. T cell precursors originate in the bone marrow but develop and mature in the thymus (T = thymic dependent). Normally, the majority of circulating lymphocytes are T cells (Kern, 2002).

1.2.1.2.5 Monocyte

Monocytes normally comprise ~3 to 8% of leukocytes. After 8 to 14 hours in the blood, they enter tissue to become tissue macrophages (also called histiocytes).

Monocytes are large cells, with abundant light gray to light blue finely granular cytoplasm. The nucleus has very finely granular chromatin and is often folded, bean shaped, or irregular. Monocytes have two functions: phagocytosis and Antigen processing and presentation (Kern, 2002).

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 haemopoietic stem cell. The megakaryocyte matures by endomitotic synchronous replication enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two (Hoffbrand and Moss, 2011).

The blood platelets are the smallest cells of the blood, averaging about 2-4 micrometers in diameter. Although much more numerous (150,000-400,000 per cubic millimeter) than the white cells, they occupy a much smaller fraction of the volume of the blood because of their relatively minute size. Like the red cells, they lack a nucleus and are incapable of cell division (mitosis), but they have a more complex metabolism and internal structure. When seen in fresh blood they appear spheroid, but they have a tendency to extrude hair like filaments from their membranes. They adhere to each other but not to red cells and white cells. Tiny granules within platelets contain substances important for the clot-promoting activity of platelets. The function of the platelets is related to hemostasis, the prevention and control of bleeding. When the endothelial surface (lining) of a blood vessel is injured, copious platelets immediately attach to the injured surface and to each other, forming a tenaciously adherent mass of platelets. The effect of the platelet response is to stop the bleeding and form the site of the developing blood clot, or thrombus. If platelets are absent, this important defense reaction cannot occur, resulting in protracted bleeding from small wounds (prolonged bleeding time).The normal resistance of capillary membranes to leakage of red cells depends on platelets. Severe deficiency of platelets reduces the resistance of the

capillary walls, and abnormal bleeding from the capillaries occurs, either spontaneously or as the result of minor injury. Platelets also contribute substances essential for the normal coagulation of the blood, and they cause a clot to shrink or retract after it has been formed. Platelets are formed in the bone marrow by segmentation of the cytoplasm (the cell substance other than the nucleus) of cells known as megakaryocytes, the largest cells of the marrow. Within the marrow the abundant granular cytoplasm of the megakaryocyte divides into many small segments that break off and are released as platelets into the circulating blood. After about 10 days in the circulation, platelets are removed and destroyed. There are no reserve stores of platelets except in the spleen, in which platelets occur in higher concentration than in the peripheral blood. Some platelets are consumed in exerting their haemostatic effects, and others, reaching the end of their life span, are removed by reticuloendothelial cells (any of the tissue phagocytes) (Rogers, 2011).

The rate of platelet production is controlled but not so precisely as the control of red cell production. A hormone like substance called thrombopoietin is believed to be the chemical mediator that regulates the number of platelets in the blood by stimulating an increase in the number and growth of megakaryocytes, thus controlling the rate of platelet production (Rogers, 2011).

1.2.2 Complete blood count test:

The complete blood count (CBC) is one of the most frequently ordered laboratory tests in the hematology laboratory (Ciesla, 2007).

Careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis. Many hematologic disorders are defined by specific findings gleaned from blood tests. Examination of hematologic parameters often yields important diagnostic information and allows broad differential diagnostic impressions to be formed, directing further, more specific testing (Greer *et al*.2003).

1.2.3 Pregnancy:

Pregnancy is the carrying of one or more off spring known as a fetus or e1`mbryo inside the womb of a female. In pregnancy, there can be multiple gestations, as in the case of twins or triplets. Childbirth usually occurs about 38 weeks after conception, in women who have a menstrual cycle length of four weeks; this is approximately 40 weeks from the last normal menstrual period (LNMP). The world health organization defines normal term for delivery as between 37 weeks and 42 weeks. For pregnancy to occur, the introduction of sperm must occur between five days before and one day after ovulation. This is because the sperm, following their ejaculation into the vagina, remain capable of fertilizing an egg for up to four to six days, and the ovulate egg remains viable for only 24 to 48 hour. Fertilization begins with fusion of sperm and egg (Widmaier, *et al*., 2006).

Only a few hundred typically sperm reach the uterine tube; the others diew along the way. In the tube, sperm move towards the oocyte as it approaches them from the opposite direction. Fertilization when it occurs, usually takes place in the uterine tubes. The fertilized egg undergoes several cell divisions and moves on to the upper portion of the uterus, where it adhere to and then embeds in the uterine wall (an event called implantation). If implantation is successful, further development of the fertilized egg continues. If fertilization does not occur, and the egg is eventually expelled from the uterus during menstruation (German and Stanfield, 2005).

During the third trimester of gestation, the dramatic growth of the fetus continues asit gains its final birth weight and its organs prepare for full function as an autonomous individual. Maternal blood volume almost doubles, and cardiac output reaches its maximum. By the 29th week, the fetus has 300 bones although many of them will fuse after birth, leaving the adult total of 206. The fetal presenting part begins to descend into the maternal pelvis in the last month of pregnancy, resulting in a decline in fundal improved respiratory and gastric function, and greater pelvic pressure and ,height discomfort. Late in this trimester changes in the cervix begin the preparations for dilation and effacement during labor and delivery (Smith, 2008).

1.2.3.1 Abortion

Abortion is the loss or failure of early pregnancy in several forms: complete, incomplete, inevitable, missed, septic, and threatened. A complete abortion is the termination of a pregnancy before the age of viability, typically defined as occurring at less than 20 weeks from the first day of the last normal menstrual period or involving a fetus of weight less than 500 g. Most complete abortions generally occur before 6 weeks or after 14 weeks of gestation. An incomplete abortion is the spontaneous passage of some, but not all, of the products of conception, associated with uniform pregnancy loss. A pregnancy in which rupture of the membranes and/or cervical dilation takes place during the first half of pregnancy is labeled an inevitable abortion. Uterine contractions typically follow, ending in spontaneous loss of the pregnancy for most patients. A missed abortion is the retention of a failed intrauterine pregnancy for an extended period; however, with ultrasound studies, this can often be detected significantly sooner than it could be on clinical grounds alone. A septic abortion is a variant of an incomplete abortion in which infection of the uterus and its contents has occurred. A threatened abortion is a pregnancy that is at risk for some reason. Most often, this applies to any pregnancy in which vaginal bleeding or uterine cramping takes place but no cervical changes have occurred (Smith, 2008).

1.2.3.2 Haematological changes during pregnancy:

Pregnancy induces a number of physiologic changes that affect the hematologic indices, either directly or indirectly. Recognizing and treating hematologic disorders that occur during pregnancy is difficult owing to the paucity of evidence available to guide consultants (Townsley, 2013).

Women often become anemic during pregnancy because of the increase in demand for iron and other vitamins in the body. It is estimated that the blood volume increases approximately 50 per cent during pregnancy, although the plasma amount is disproportionately greater. This causes dilution of the blood, making the hemoglobin

concentration fall, with hemoglobin concentration at its lowest between weeks 25 and 30 (Salhan, *et al*. 2012).

During pregnancy, the total blood volume increases by about 1.5 l, mainly to supply the needs of the new vascular bed (Pavord and Hunt, 2010).

In pregnancy, plasma volume increases 25%–80% between the sixth and twenty-fourth week of gestation. However, the increase in RBC mass has been found to be approximately 30% between the twelfth and thirty-sixth week of gestation when iron and folate are supplemented. The discrepancy between the rate of increase in plasma volume and that in RBC mass leads to physiological anemia. In late pregnancy, plasma volume increases at a slower rate, inducing a slight rise in hematocrit level. These physiological changes during pregnancy make it difficult to define normal hematological reference intervals for pregnant women (Akinbami, 2013).

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) hematocrit (HCT) value is likewise lower in pregnancy (Pavord and Hunt, 2010).

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 B12 and folate (Pavord and Hunt, 2010).

Previous studies have reported that pregnancy is usually accompanied by leukocytosis, but the full sequential changes of the various cell types responsible for this observed leukocytosis have not been clearly determined in all geographical locations and physiological conditions (Akinbami, 2013).

In non pregnant patients a normal WBC count is somewhere between 5 and 10 (5,000–10,000 cells/mm^3), but for pregnancy those normal values can be between 6 and 16(6×10^9/L –16×10^9/L). in the third trimester and may reach 20 to 30 in labor and early postpartum (Derricot and Cartwright, 2013). In the hours after delivery3, healthy women have been documented as having WBC (9×10^9/L –2×10^9/L). By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (4×10^9/L–10×10^9/L). Neutrophils contribute most to the overall higher WBC (Pavord and hunt, 2010).

Lymphocyte count decreases during pregnancy through first and second trimesters and increases during the third trimester, but remains low in the early puerperiumas compared to normal non-pregnant values. Detailed studies of T and B lymphocyte subsets in peripheral blood and the proliferative responses of these cells to mitogens found more helper and suppressor cells and less killer cell during pregnancy. Lymphocyte proliferation in response to a variety of agents was found to be impaired in pregnancy, suggesting that there is an immunosuppressant factor present in the serum. Monocyte count is higher in pregnancy, especially in the first trimester, but decreases as gestation advances.4 Typical values3,4 in the third trimester are 0.2×10^9–1.0×10^9/L, as compared to non-pregnant values 0.1×10^9–0.9×10^9/L. The monocyte to lymphocyte ratio is markedly increased in pregnancy. Eosinophil and basophil counts do not change significantly during pregnancy (Pavord and Hunt, 2010).

Normal pregnancy is associated with a physiologic fall in the platelet count that is characterized by a left shift in the platelet count distribution. The reason for this decline is unknown, although it has been speculated that these changes may reflect dilution, decreased platelet production, or increased platelet turnover during pregnancy. Regardless, the fall in the platelet count during normal pregnancy results in some pregnant women developing platelet counts that falls into the thrombocytopenic range. Generally, these individuals have mild thrombocytopenia that first becomes apparent in the mid-second to third trimester of pregnancy (McCrae, 2010).

The PLT count is slightly lower in pregnant than in non-pregnant women. Most studies report an approximate 10% lower PLT level at term compared with at pre-pregnancy. The majority of pregnant women still have levels within the normal range; however, if the pre-pregnancy level is border line or there is a more severe reduction, this may fall below the normal range. The mechanisms for this are thought to be due to dilution effects and accelerated destruction of PLTs passing over the often scarred and damaged trophoblast surface of the placenta. PLT counts may also be lower in women with twin compared with single pregnancies, possibly due to greater thrombin generation. Although most cases of thrombocytopenia in pregnancy are mild, with no adverse outcome for mother or baby, occasionally a low PLT count may be part of a complex disorder with significant morbidity and be (rarely) life-threatening (Akinbami, 2013).

1.2.3.3 Common types of Anaemia during pregnancy:

The most frequent hematologic complication during pregnancy is anemia. The World Health organization defines anemia in pregnancy as hemoglobin below 11 g/dl (Townsley, 2013).

Anemia in pregnancy is defined by the World Health Organization (WHO) as a hemoglobin concentration below 11 g/dL. It continues to be a major health problem in many developing countries and is associated with increased rates of maternal and prenatal mortality, premature delivery, low birth weight, and other adverse outcomes. More than half of the pregnant women in the world have hemoglobin levels indicative of anemia. Although only 15% of pregnant women are anemic in developed countries, the prevalence of anemia in developing countries is relatively high (33% to 75%).The most common cause of anemia in pregnancy (Dim, 2007).

A number of normal physiologic processes occur during pregnancy leading to the term “physiologic anemia of pregnancy”. The plasma volume increases (40–50%) relative to red cell mass (20–30%) and accounts for the fall in hemoglobin concentration (Townsley, 2013).

1.2.3.3.1 Iron deficiency Anaemia

Iron deficiency anemia is the most common health problem that women face worldwide. It affects about 20%of the world's population and is a significant cause of morbidity and mortality. Of anemias diagnosed in pregnancy, 75% are due to iron deficiency, (Pavord and Hunt 2010).

Up to 600 mg iron is required for the increase in red cell mass and a further 300 mg for the fetus. Despite an increase in iron absorption, few women avoid depletion of iron reserves by the end of pregnancy (Hoffbrand and Moss, 2011).

However, if the hemoglobin falls below 11 gm/dL an evaluation for iron deficiency anemia (IDA) should be initiated since iron deficiency is responsible for the majority of anemias diagnosed during pregnancy. The increased demand on the bone marrow requires women to increase their daily iron intake from 18 mg per day to 27 mg per day. An association between severe anemia (hemoglobin <9 gm/dL) and poor pregnancy outcome has been reported by multiple observational studies triggering the recommendation for universal iron supplementation at a dose equal to the Recommended Dietary Allowance. Although prophylactic supplementation is controversial, the practice has been shown to increase gestation duration and increase infant birth weights compared to non-supplemented women. The risk of adverse pregnancy outcomes is highest when maternal anemia is detected early during pregnancy (first trimester) possibly owing to the difficulty in distinguishing physiologic anemia from IDA in late pregnancy (third trimester)(Townsley, 2013).

The dietary bioavailability of iron depends on the iron content of the food and its form. Heme iron, derived from meat is more readily absorbed than non-heme iron. Absorption is facilitated by reducing agents such as vitamin C, hence the recommendation to take iron supplements with orange juice or ascorbic acid tablets. Absorption is inhibited by phytates in cereals, tannins in tea and polyphenols in some vegetables. Only approximately 10% of dietary iron is absorbed. This increase in pregnancy and triples from the first to the third trimester peaking after 30 weeks (Pavord and Hunt, 2010).

Most women do not have adequate iron stores for pregnancy secondary to chronic blood loss from menstruation, and some may not tolerate oral iron therapies due to impaired ingestion or side effects further increasing their risk for IDA (Townsley, 2013).

Iron deficiency develops sequentially, with storage iron becoming depleted initially. This is followed by a fall in the amount of iron available for erythropoiesis. Subsequently, the peripheral blood hemoglobin drops and, with that, there is a fall in the delivery of oxygen to peripheral tissues, and the patient develops clinical symptoms and signs. Patients with iron deficiency are often asymptomatic, but symptoms may occur without an anemia. Iron dependent enzymes in every cell are affected and there are neuromuscular, gastrointestinal and epithelial consequences. Prior to the development of an anemia, the signs and symptoms of iron deficiency are non-specific and include reduced exercise tolerance and tiredness. Severe iron deficiency is associated with pallor, glossitis, angular chelitis, nail ridging, and when severe nail spooning – koilonychia. Dysphasia can develop if a post-cricoid web occurs. Iron deficiency can also affect cellular immunity and phagocytosis, with women being increasingly susceptible to infection. Pica can occur in as many as 50% of patients as a symptom of severe iron deficiency and can take different forms – craving for earth, clay, starch, and ice. It improves with iron replacement (Pavord and Hunt, 2010).

1.2.3.3.2 Megaloblastic Anaemia

The megaloblastic anemias due to folic acid deficiency, and to a lesser extent vitamin B12 deficiency, can also be a cause of anemia during pregnancy (Townsley, 2013).

Folate deficiency has a prevalence of less than 5% in developed countries and a very low prevalence where there is food fortification with folic acid. Worldwide folate deficiency is far more common and may complicate one-third of pregnancies. It is a reflection of nutritional Status (Pavord and Hunt, 2010).

Folate requirements are increased approximately two fold in pregnancy and serum folate levels fall to approximately half the normal range with a less dramatic fall in red cell folate. In some parts of the world, megaloblastic anaemia during pregnancy is common because of a combination of poor diet and exaggerated folate requirements. Given the protective effect of folate against neural tube defects (NTDs) as well as against anaemia, 400 μ g/day folic acid (5 mg if there has been a previous NTD pregnancy) should be taken periconceptually and throughout pregnancy. Food fortifi cation with folate is now being practised in many countries (not the UK) and has been associated with a fall in incidence of NTDs. Vitamin B 12 deficiency is rare during pregnancy although serum vitamin B 12 levels fall to below normal in 20 – 30% of pregnancies and low values are sometimes the cause of diagnostic confusion (Hoffbrand and Moss, 2011).

Folate is awater-soluble vitamin. It cannot be synthesized by humans, but is found in a wide variety of food sources, including leafy green vegetables, liver, citrus fruits, nuts, bread, and dairy produce. Folate is heat labile and it is often lost in the cooking process. It is absorbed mainly in the jejunum and then taken up by the liver. Folate stores last several months (Pavord and Hunt, 2010).

Vitamin B12 deficiency is most likely to occur in the third trimester, particularly in women with inadequate diets .Vitamin B12 is involved in the folate pathway in the formation of DNA, which is essential for cell multiplication during pregnancy that is required for foetal development. Hyperhomocysteinemia, which is caused by low vitamin B12 levels, is associated with many clinical conditions, for example, placental vasculopathy, which has an effect on foetal growth (Akhtar and Hassan, 2012).

B12 and/or folate deficiency cause a megaloblastic anemia. This is usually suspected by the presence of macrocytic red cells. Megaloblastic erythropoiesis requires a bone marrow to demonstrate large developing red cells with nuclear cytoplasmic asynchrony and giant metamyelocytes. In practice, this is rarely necessary. In pregnancy, interpretation of MCV can be more difficult due to the physiological

increase in red cell size and the increased likelihood of an additional iron deficiency anemia that may reduce the MCV (Pavord and Hunt, 2010).

Blood film examination can provide useful diagnostic clues. Features suggestive of a megaloblastic anemia include hyper-segmented neutrophil nuclei (more than five segments), oval macrocytes and mild leukopenia, and thrombocytopenia in severe cases. If iron deficiency co-exists with a megaloblastic anemia, the blood film will be dimorphic with a mixture of large and small red cells (macrocytic and microcytic cells).Red cell folate assays give an indication of overall body tissue levels and are better than serum folate levels that are affected by recent diet and fluctuate significantly from day to day. Homocysteine is the precursor to methionine in the remethylation cycle and increases in B12 or folate deficiency, as both are required as co factors. This indirect measurement is a sensitive marker for folate deficiency. Megaloblastic erythropoiesis is demonstrated by the finding of large erythroblasts and giant abnormally shaped metamyelocytes.B12 assays give an indication of overall body tissue levels.B12 levels fall in pregnancy, but this is not thought to represent a true tissue deficiency. Intrinsic factor antibodies can be helpful in the diagnosis of pernicious anemia. Schilling test, this test has been used classically to diagnose pernicious anemia. It is contradicted in pregnancy because of the radiation risks. A therapeutic trial of B12 can confirm the diagnosis. Reticulocytosis occurs within 3–4 days and peaks at day 6–7 (Pavord and Hunt, 2010).

1.2.4 Previous study:

Many researches were conducted in the world to evaluate hematological profile in normal pregnancy. Study done by Shen, *et al*. (2010), which aimed to investigate the haematological profile during pregnancy in Chinese women and compared these to the established values for white and black women. The results showed Hb concentration, RBC count, HCT and PLT count were lower during pregnancy.

In Africa study done by Akingbola, *et al.*(2006), The result showed lowering in Hb and HCT and platelet and increase WBCs.

Other study done by Melku, *et al.* (2005) in Ethiopia showed the prevalence of anemia was high especially in third trimester; mild type and normocytic normochromic anemia was dominant.

In Sudan study conducted by Elgari (2013),The results showed that there were significant decreased in RBCs count, hemoglobin (Hb) and packed cell volume (PCV) of pregnant women compared to non pregnant women (P value <0.05) and significant decrease in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pregnant women (P value <0.05). TWBCs count was increased significantly (P. value < 0.050) in contrast to platelets count which was significantly lower than the normal control (P. value <0.05).

Other study conducted by Abdelgader, *et al*(2014) which aimed to measure Haemoglobin level, RBCs Indices, and iron status in pregnant females in Sudan, the results showed that out of 80 pregnant females, 8 (10%) of them had low Hb level, while 72 (90%) had normal Hb level. RBC indices showed 62 (77.5%) mothers had normal MCV, while 18 (22.5%) mothers had low MCV. 63 (78.8%) had normal MCH, while 17 (21.2%) had low MCH, 78 (97.5%) had normal MCHC, while 2 (2.5%) had low MCHC.

A study done by Farah and Munsoor (2012) showed there was statistically significant difference in TWBCs, RBCs, Hb, HCT, MCV, MCH and serum ferritin level among study group when compared to control group. In addition there was significant decrease in mean of PLT in the third trimester.

Study done in eastern Sudan by Adam, *et al*. (2005) the result showed that (62.6%) of pregnant women had anemia.

1.3 Rationale

In Sudan, studies concerning the hematological status of pregnant women are limited. Pregnant women may be subjected to pregnancy complications which threatened her life and the fetus, so hematological screening are highly needed throughout the pregnancy period. The results of this study may add new data to help researchers in the future to create laboratory records of hematological profile of Sudanese pregnant women.

1.4 Objectives

1.4.1General objective

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

1.4.2 Specific objectives

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

-To assess the effect of abortion, age and numbers of children on pregnant women at third trimester.

Chapter Two

Materials and Methods

2.1 Study Design and Study area:

This is a case control study aimed to determine complete blood count of Sudanese pregnant women at third trimester attending Khartoum Hospitals during the period between April and October 2015.

2.2 Sample Size:

Eighty pregnant women and 40 non-pregnant women were enrolled in this study.

2.3 Inclusion Criteria:

Pregnant woman with apparently normal pregnancy and without diseases that may cause haematological changes.

2.4 Exclusion Criteria:

Presence of any diagnostic disease that interfere with the variables under study.

2.5 Ethical consideration:

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

2.6 Tools of Data Collection:

Data was collected using structural interviewing questionnaire and blood sample

2.7 Method of sample collection:

2.7.1 Requirements:

1- Automated Haematological Analyzer (Mindary BC-3000 plus) for determination of complete blood count.

2- EDTA tube container.

3- Alcohol swab (70% Alcohol (ethanol)).

4- Cotton.

5- Tourniquet.

6- Blister.

2.7.2 Procedures of sample collection:

1- A pregnant women either sat or lid on an examination table

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

3- The skin was cleaned with 70% ethanol and allowed to dry.

4- A tourniquet was applied to the arm, tight sufficiently to distend the vein, but not tightly to cause discomfort.

5- 3ml of blood samples were taken from the superficial vein of the forearm.

6- Blood sample was collected in K_2EDTA (1.2 mg/ml of blood

7- Personal details were checked up on the forms and blood vials (Khalil, 2013).

2.8 Procedure of CBC:

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

2.8.1. Hemoglobin concentration (HGB or HB)

Automated counter used nonhazardous chemical, such as sodium lauryl sulphate, imidazole, and sodium dodecyl sulphate or dimethyl lauryl amineoxide. 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, 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).

2.8.2 Red blood cell count (RBC)

Red cells and other blood cells were counted in systems based on aperture impedance or light-scattering technology. Normal range in women $4.3 \pm 0.5 \times 10^{12}$/L (Dacie and Lewis, 2011) .

2.8.3. Platelet count

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. Platelet normal range in women $280 \pm 130 \times 10^{9}$/L (Dacie and Lewis, 2011).

2.8.4. Packed cell volume (PCV)

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

2.8.5. Red cell indices

2.8.5.1. Mean cell volume (MCV)

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

2.8.5.2 Mean corpuscular hemoglobin (MCH)

MCH is derived from the Hb divided by RBC. Women normal range29.5 ± 2.5 pg (Dacie and Lewis, 2011).

2.8.5.3. 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).

2.8.6. 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).

2.8.7. 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).

2.9 Quality control

Quality Control (QC) consists of strategies and procedures that measure the precision and stability of the analyzer. The results imply the reliability of the sample results. QC involves measuring materials with known, stable characteristics at frequent intervals. Analysis of the results with statistical methods allows the inference that sample results are reliable. Mindray recommends you run the QC program daily with low, normal

and high level controls. A new lot of controls should be analyzed in parallel with the current lot prior to their expiration dates.

2.10 Statistical analysis:

Data were entered into computer and analyzed by SPSS used independent test. P significant level was set at $P \leq 0.05$

Chapter Three

Results

This study aimed to determine CBC during pregnancy of Sudanese pregnant women at third trimester compared to non-pregnant women attending Khartoum Hospitals.

There was a significant decrease in total red blood cells, Hb and MCHC in pregnant women when compared with non-pregnant women. MCV increased significantly in test compared to non-pregnant women. HCT and MCH showed no significant differences. (Table 3.1)

Table (3.2) showed that lymphocyte and platelet of pregnant women significantly decreased compared to non-pregnant women. Neutrophil and MXD count significantly increased in pregnant women compared to non-pregnant women. No significant differences in percentage of MXD and platelets count in pregnant women when compared with control.

No significant differences in CBC according to history of abortion and according to number of children (tables 3.3, 3.4, 3.5 and 3.6).

According to tables (3.7 and 3.8) MCHC was significantly decreased in women with age less than 30 years (P= 0.03) and no significant difference in other haematological parameters according to the age group**.**

Table (3.1) Total Red Blood Cells count, Hb, HCT and RBCs indices of pregnant women compared to non-pregnant women

Variable	**Sample**	**No**	**Mean± Std. deviation**	**P value**
TRBCs $\times 10^{12}$ /l	Pregnant	80	4.0± 0.46	0.000
	non-pregnant	40	4.3± 0.38	
Hb g/dl	Pregnant	80	10.9 ±1.24	0.000
	non-pregnant	40	12.2 ±1.07	
HCT%	Pregnant	80	33.3 ±3.15	0.627
	non-pregnant	40	33.6±2.60	
MCV fl	Pregnant	80	84.6±5.91	0.000
	non-pregnant	40	78.5±6.81	
MCH pg	Pregnant	80	27.4±2.65	0.079
	non-pregnant	40	28.4±2.99	
MCHC %	Pregnant	80	32.4±1.72	0.000
	non-pregnant	40	36.1±2.07	

Table (3.2) Total White Blood cells count, differential Percentage and absolute and platelets of pregnant women compared to non-pregnant women

Variable	**Sample**	**No**	**Mean±Std. deviation**	**P value**
TWBCs $\times 10^9$ /l	Pregnant	80	7.6± 1.84	0.144
	non-pregnant	40	6.9 ±2.62	
Neutrophil $\times 10^9$ /l	Pregnant	80	4.9 ±1.49	0.020
	non-pregnant	40	4.1 ±2.59	
Lymphocyte $\times 10^9$ /l	Pregnant	80	1.9 ± 0.59	0.001
	non-pregnant	40	2.3 ± 0.66	
MXD$\times 10^9$ /l	Pregnant	80	0.6 ± 0.32	0.044
	non-pregnant	40	0.4 ± 0.38	
Neutrophil%	non-pregnant	80	66.0 ±8.05	0.000
	non-pregnant	40	55.0 ±16.74	
Lymphocyte %	Pregnant	80	26.7 ±8.02	0.000
	non-pregnant	40	37.9 ±13.85	
MXD %	Pregnant	80	7.9 ±3.41	0.520
	non-pregnant	40	7.3 ±5.46	
PLT $\times 10^9$ /l	Pregnant	80	223 ±61.0	0.000
	non-pregnant	40	292 ±74.88	

Table (3.3) Effect of history of abortion on TRBCs count Hb, HCT and RBCs indices

Variable	**Abortion**	**No**	**Mean± Std. deviation**	**P value**
TRBCs $\times 10^{12}$ /l	yes	23	3.9± 0.44	0.702
	no	57	4.0± 0.46	
Hb g/dl	Yes	23	10.9±1.08	0.977
	No	57	10.9±1.30	
HCT %	Yes	23	33.1 ±2.75	0.765
	No	57	33.4 ±3.31	
MCV fl	Yes	23	84.9 ±4.72	0.739
	No	57	84.5 ±6.35	
MCH pg	Yes	23	27.9 ±2.12	0.232
	No	57	27.2 ±2.82	
MCHC %	Yes	23	32.7 ±1.24	0.178
	No	57	32.2 ±1.87	

Table (3.4) Effect of history of abortion on TWBCs count, differential percentage and absolute and platelets

Variable	Abortion	No	Mean±Std. deviation	P value
TWBCs $\times 10^9$ /l	Yes	23	7.7± 1.55	0.592
	No	57	7.5± 1.95	
Neutrophil $\times 10^9$ /l	Yes	23	4.9± 1.30	0.987
	No	57	4.9± 1.57	
Lymphocyte $\times 10^9$ /l	Yes	23	1.9± 0.48	0.996
	No	57	1.9± 0.64	
MXD$\times 10^9$ /l	Yes	23	0.6± 0.21	0.889
	No	57	0.6± 0.36	
Neutrophil%	Yes	23	65.9±6.26	0.908
	No	57	66.1± 8.72	
Lymphocyte %	Yes	23	26.3± 6.08	0.776
	No	57	26.8± 8.72	
MXD %	Yes	23	8.2± 2.44	0.649
	No	57	7.8± 3.74	
PLT $\times 10^9$ /l	Yes	23	229± 72.0	0.607
	No	57	220± 56.6	

Table (3.5) TRBCs count Hb, HCT and RBCs indices according to numbers of children

Variable	No of children	No	Mean±Std. deviation	P value
TRBCs $\times 10^{12}$ /l	<2	41	4.03± 0.44	0.356
	>2	39	3.9± 0.48	
Hb g/dl	<2	41	11.1± 1.26	0.383
	>2	39	10.8± 1.23	
Hct %	<2	41	33.6±3.30	0.307
	>2	39	32.9±2.98	
MCV fl	<2	41	84.6± 5.92	0.978
	>2	39	84.6± 5.97	
MCH pg	<2	41	27.5± 2.92	0.814
	>2	39	27.3± 2.36	
MCHC %	<2	41	32.4± 1.96	0.664
	>2	39	32.3± 1.45	

Table (3.6) TWBCs count, differential Percentage and absolute and Platelets according to numbers of children

Variable	**No of children**	**No**	**Mean±Std. deviation**	**P value**
TWBCs $\times 10^9$ /l	<2	41	7.6± 1.79	0.932
	>2	39	7.6± 1.90	
Neutrophil $\times 10^9$ /l	<2	41	5.0± 1.44	0.646
	>2	39	4.8± 1.54	
Lymphocyte $\times 10^9$ /l	<2	41	1.9± 0.55	0.303
	>2	39	2.0± 0.64	
MXD$\times 10^9$ /l	<2	41	0.5 ± 0.23	0.264
	>2	39	0.6± 0.38	
Neutrophil%	<2	41	67.5± 7.69	0.095
	>2	39	64.4± 8.22	
Lymphocyte%	<2	41	25.3± 7.31	0.118
	>2	39	28.1± 8.45	
MXD %	<2	41	7.3± 2.49	0.108
	>2	39	8.6± 4.09	
PLT $\times 10^9$ /l	<2	41	227 ± 61.22	0.490
	>2	39	218 ± 61.3	

Table (3.7) TRBCs count Hb, HCT and RBCs indices according to the age group

Variable	**Age**	**No**	**Mean±Std. deviation**	**P value**
TRBCs $\times 10^{12}$ /l	<30	50	4.0± 0.42	0.56
	>30	30	3.9± 0.51	
Hb g/dl	<30	50	10.8± 1.28	0.44
	>30	30	11.1± 1.17	
HCT %	<30	50	33.3± 3.23	0.86
	>30	30	34.0± 3.06	
MCV fl	<30	50	84.1± 6.29	0.35
	>30	30	85.3± 5.22	
MCH pg	<30	50	27.1± 2.92	0.11
	>30	30	27.9± 2.04	
MCHC %	<30	50	32.1± 1.79	0.03
	>30	30	32.9± 1.48	

Table (3.8) TWBCs count, differential Percentage and absolute and Platelets according to the age group

Variable	Age	No	Mean±Std. deviation	P value
TWBCs $\times 10^9$ /l	<30	50	7.6± 1.78	0.870
	>30	30	7.5± 1.95	
Neutrophil $\times 10^9$ /l	<30	50	4.9± 1.45	0.645
	>30	30	5.1± 1.57	
Lymphocyte $\times 10^9$ /l	<30	50	1.9±0.55	0.960
	>30	30	1.9± 0.67	
MXD$\times 10^9$ /l	<30	50	0.6± 0.24	0.564
	>30	30	0.6± 0.41	
Neutrophil%	<30	50	65.9± 7.31	0.846
	>30	30	66.3± 9.27	
Lymphocyte%	<30	50	26.6± 7.31	0.923
	>30	30	26.8± 9.20	
MXD %	<30	50	7.6± 2.78	0.373
	>30	30	8.4± 4.25	
PLT $\times 10^9$ /l	<30	50	227 ± 63.67	0.437
	>30	30	216 ± 56.79	

Chapter Four

Discussion, Conclusion, and Recommendations

4.1. Discussion

This study aimed to measure of CBC of pregnant women at third trimester.

The results of this study showed that there was a significant decrease in Hb, TRBCs, MCHC (p=0.00) in pregnant women when compared with non-pregnant women. These results agreed partly with the results of a study in China which showed that during pregnancy Hb concentration, TRBC count, HCT were lower (Shen, 2010). Akinbami *et al*. (2013) in Lagos showed that HCT in pregnant women at third trimester was 33.04% ± 3.88%, Hb concentration value was 10.38 ± 1.27 g/dL. In Sudan there were significant decrease in RBCs count, Hb and HCT of pregnant women compared to non pregnant women (Elgari, 2013). Adam (2005) in eastern Sudan showed that (62.6%) of pregnant women had anaemia (Hb <11g/dl). Akingbola *et al*. (2006) in South Nigeria showed that pregnancy is characterized by lowest values of haemoglobin, hematocrit.

Women often become anemic during pregnancy because of the increase in demand for iron and other vitamins in the body. It is estimated that the blood volume increases approximately 50 per cent during pregnancy, although the plasma amount is disproportionately greater. This causes dilution of the blood, making the hemoglobin concentration fall, with hemoglobin concentration at its lowest between weeks 25 and 30 (Salhan, *et al*. 2012).

During pregnancy, the total blood volume increases by about 1.5 l, mainly to supply the needs of the new vascular bed (Pavord and Hunt, 2010).

MCV was a significantly increase, these result agreed with Akingbola *et al*. (2006) in South Nigeria that showed MCV in the pregnant women was slightly raised and disagreed with Elgari (2013) which showed significant decrease in MCV. In un

complicated pregnancy, the (MCV) typically rises by approximately 4 fL (Hoffbrand and Moss 2011).

The result of this study showed no significant difference in HCT and MCH.

Platelets count and lymphocytes decreased significantly (p= 0.00) in pregnant women when compared with non-pregnant women. These results agreed with the results of the study in China, which showed PLT count was lower (Shen, *et al*, 2010). Also Akinbami, *et al*. (2013) showed that PLT was lower. Platelets count significantly lower than the normal control (Elgari, 2013). A study by Pavord and Hunt (2010) has showed that the platelet count decreases during pregnancy, particularly in the third trimester.

The PLT count is slightly lower in pregnant than in non-pregnant women. The mechanisms for this are thought to be due to dilution effects and accelerated destruction of PLTs passing over the often scarred and damaged trophoblast surface of the placenta (Akinbami, 2013). The platelet count typically falls by approximately10% in an uncomplicated pregnancy. (Hoffbrand and Moss 2011). Lymphocyte proliferation in response to a variety of agents was found to be impaired in pregnancy, suggesting that there is an immunosuppressant factor present in the serum (Pavord and Hunt 2010).

Lymphocyte proliferation in response to a variety of agents was found to be impaired in pregnancy, suggesting that there is an immunosuppressant factor present in the serum (Pavord and Hunt 2010). There was a significant increase in MXD absolute count and neutrophils ($p \leq 0.04$) of pregnant women more than non –pregnant women

Previous studies have reported that pregnancy is usually accompanied by leukocytosis, but the full sequential changes of the various cell types responsible for this observed leukocytosis have not been clearly determined in all geographical locations and physiological conditions (Akinbami, 2013).

There was no significant difference in haematological parameters according to history of abortion (tables 3.3and 3.4). These findings disagreed with the results by Khalil (2012) who indicated that no significant differences between positive and negative history of abortion.

In tables (3.5 and 3.6) there was No significant difference in CBC according to number of children. According to age of pregnant women MCHC significantly increased in pregnant women with age more than 30 years (P= 0.03) in > 30 more than < 30 years and no significant difference in other parameters (tables 3.7 and 3.8).

Khalil (2012) showed that no significant affect of history of abortion and number of pregnancy on CBC.

4.2 Conclusions

1. TRBCs, Hb and MCHC were significantly decreased but MCV was significantly increased in pregnant women at third trimester when compared to non-pregnant women.

2. Neutrophil and MXD significantly increase in pregnant women but significant decrease in lymphocyte and platelet count.

3. No significant differences in CBC in pregnant women according to history of abortion and number of children.

4. MCHC was significantly decrease in pregnant women with age less than 30 years but no significant differences in other haematological parameters of study group with different age groups.

4.3. Recommendations

1. Routine hematological tests particularly TRBCs, Hb, MCV, MCHC, TWBCs and differential count are important. So that pregnancy complications could be detected and managed.
2. More special care to pregnant women at third trimester which improves her health, to meet fetus requirements.
3. Statistical baseline data of hematological status of Sudanese pregnant women is highly needed.

References

Abdelgader E. A., DiabT. A., Kordofani A. A., Sana E. And Abdalla S. E. (2014) Haemoglobin level, RBCs Indices, and iron status in pregnant females in Sudan. Basic Research Journal of Medicine and Clinical Sciences. 3 (2):8-13.

Adam I., Khamis A.H., Ebashir M. I. (2005). Prevalence and risk factors for anaemia in pregnant women of eastern Sudan Pub Med, 99(10) : 739-43.

Akhtar M. and Hassan I. (2013). Severe Anaemia during Late Pregnancy. Case Reports in Obstetrics and Gynecology. 2012; 2012: 485452.

Akinbami A.A., Ajibola S. O., Rabiu K. A., Adewunmi A. A., Dosunmu A. O., Adediran A, Osunkalu V. O., Osikomaiya B. I., and Ismail K. A. (2013). Hematological profile of normal pregnant women in Lagos, Nigeria. International Journal of Women Health.5: 227–232.

Akingbola T. S.,Adewole I.F., Adesina O.A., Afolabi K.A., Fehintola F.A., Bamgboye E.A., Aken'ova Y.A., Shokunbi W.A., Anwo J.A., Nwegbu M.M.. (2006). Haematological profile of healthy pregnant women in Ibadan, south-western Nigeria. Journal of Obstetric and Gynaecoogyl. 26(8):763-9.

Ayokunle O.,(2011). Hematological profile of pregnant women in southwest of Nigeria. Asian pacific journal of tropical disease. 1(3):232-234.

Ciesla B.(2007). Hematology in Practice. USA. F. A. Davis Company.

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

Derricott B., Cartwright C. (2013). Pregnancy, Physiologic Changes and Laboratory Values. Wild Iris Medical Education.(707) 937-0518.

Dim C. C. (2007). The Prevalence of Anemia Among Pregnant Women at Booking in Enugu, South Eastern Nigeria. PubMed Central. 9(3): 11.

Dim C.C. , Ugwu E.O. , Dim N.R. , and Anyaehie U.B.(2015). Hematocrit, Anemia, and Arm Preference for Blood Sample Collection: A Cross-Sectional Study of Pregnant Women in Enugu, South-Eastern, Nigeria. Annals of Medical and Health Sciences Research. 5(1): 36–41.

Elgari M. (2013). Evaluation of Hematological Parameters of Sudanese Pregnant Women attending at Omdurman Al Saudi Maternity Hospital.Egyptian Academic Journal of Biological Science. 5 (1): 37 – 42.

Estridge B. H., Renolds. A. P, walters N.J.(2000). Basic medical laboratory technique. 4th Edition, Delmar puplisher.

Farah A.M., Munsoor M.M. (2012). Status of iron Deficiency Anemia among Sudanese pregnant women referred to Khartoum Teaching Hospital sustech. Edu / handle/ 123456789/1504.

German W.J. and Stanfield C.I. (2005). Principle of human physiology.2nd edition. United State. Dary Fox.

Greer J. P., Foerster J. Lukens J. N. (2003). Wintrobe's Clinical Hematology, 11th Edition, Lippincott Williams & Wilkins Publishers.

Grimes D. A., Stuart, G. (2010). Abortion jabberwocky: the need for better terminology". Contraception **81** (2): 93–6.

Hoffbrand A. V. and Moss P.A.H,(2011). Essential Haematology, 6th edition. London.Wiley_Blackwell.

Kern W. F. (2002), PDQ Hematology, 1st edition, Canada . BC Deker Inc.

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

McCrae K. R. (2010). Thrombocytopenia in Pregnancy. American Society of Hematolog.2010(1) :397-402.

Melku M., Addis Z. , Alem M. and Enawgaw B (2005). Prevelence and Predictors of Maternal Anemia during pregnancy in Gondar, Northwest Ethiopia: An Institutional Based Cross-Sectional study. Anemia. Vol 2014(2014).

Paidas M. J., Hossain N, ShamsiT S , Rodger M A, langhoff-Roos J, lockwood C J, (2012). Hemostasis and thrombosis in obstetrics and gynecology,1stEdition, black well.

Pavord S. and Hunt B. (2010). Obstetric Hematology manual, New York. Cambridge university press.

Rogers K. (2011). The Human Body Blood physiology and circulation, 1stEdition, New York. Britannica educational publishing.

Salhan S., Tripathi V.,Singh R. and Gaikwad H.S . (2012). Evaluation of Hematological Parameters in Partial Exchange and Packed Cell Transfusion in Treatment of Severe Anemia in Pregnancy. Anaemia, vol. 2012, Article ID 608658, 7 pages, 2012.

Shen C., Jiang Y. M., Shi H., Liu J. H., Zhou W. J., Dai Q. K. and Yang H. (2010). A prospective, sequential and longitudinal study of haematological profile during normal pregnancy in Chinese women. Journal of Obstetrics and Gynaecology **.** 4 (5) P. 357-361.

Smith R. P.,(2008). Netter's obstetrics and gynecology. 2nd edition. Saunders, an imprint of Elsevier Inc.

Townsley D .M. (2013). Hematologic Complications of Pregnancy, PMC Journals. 50(3): 222–231.

Widmaier E.P., Raff H. and Strang K.T (2006). Vander's Human Physiology, The mechanisms of body function. 10th edition. New York MC Graw-Hill.

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

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

Name……..Age……

…….Occupation…….……Husband occupation……

Month of pregnancy……..… No of pregnancy……

Abortion: yes ( ) No ( )

Suffer from disease: Malaria ( ) Anemia ( ) Typhoid ( ) other……Previous

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

Result:

WBC….....RBC……HGB……..…..……

HCT……....MCV……..MCH……..……..

MCHC……NEUT#……..….NEUT%.....LYM#

……...LYM%.....MXD#……...

MXD%.....PLT…….

Appendix (2)

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم ومبحث المناعة الدموية

إقرار موافقة بالمشاركة

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)

Principle of Mindary

General principle:

The two independent measurement methods used in this analyzer are:

- the Coulter method for determining the WBC, RBC, and PLT.
- the colorimetric method for determining the HGB.

During each analysis cycle, the sample is aspirated, diluted and mixed before the determination for each parameter is performed.

WBCs, RBC/PLT measurement

WBCs, RBCs/PLTs are counted and sized by the Coulter method. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in a conductive diluents as it passes through an aperture of known dimensions.

An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated signals the number of particles that passed through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each pulse is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the WBC threshold, it is counted as a WBC. If the pulse generated is above the RBC/PLT lower threshold; it is counted as a RBC/PLT

Hb measurement

Hb is determined by the colorimetric method.

The WBC/Hb dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is

measurable at 525 nm. An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then is measured by a photo-sensor that is mounted on the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading readings taken when there is only diluent in the bath). The Hb is calculated per the following equation and expressed in g/L.

Hb (g/L) = Constant×Log_{10} (Blank Photocurrent/Sample Photocurrent)

MCV, HCT, MCH,and MCHC:

Based on the RBC histogram, this analyzer calculates the mean cell volume (MCV) and expresses the result in fL .

This analyzer calculates the HCT (%),MCH (pg) and MCHC (g/L) as follow

$$\mathrm{HCT} = \frac{\mathrm{RBC} \times \mathrm{MCV}}{10}$$

$$\mathrm{MCH} = \frac{\mathrm{Hb}}{\mathrm{RBC}}$$

$$\mathrm{MCHC} = \frac{\mathrm{Hb}}{\mathrm{HCT}}$$

Where the RBC is expressed in 10^{12}/L, MCV in fL and Hb in g/L.

Figure (2.1) Mindary BC-3000 plus