Measurement of Complete Blood Count among Sudanese Pregnant Women attended in Military Hospital Omdurman

قياس تعداد الدم الكامل في النساء السودانيات الحوامل في مستشفى السلاح الطبي امدرمان

Dissertation submitted in partial fulfillment of the Requirement of M.SC Degree in Hematology.

BY

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Supervisor:

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2015
آَوْهُ يَا لَهَّ مِنْ الْشَّيْطَانِ الْجَحِيمِ

فَأَطْمَسَ١٣ وَلَقَدْ خَلَقْنَا الْإِنسَانَ مِنْ سَلْطَانٍ دَوَنَ طَيِّنٍ١٤ ثُمَّ جَعَلْنَاهُ نُطْفَةً فِي قَرْرٍ مُّكَبِّرٍ١٥

فَخُلَقْنَا النَّطْفَةَ عَلَقَةً فَخَلَقْنَا الْعَلَقَةَ مُضَفَّكَةً فَخَلَقْنَا الْمُضَفَّكَةَ عَطُدًا١٦

فَكَسَوْا الْوُزْنَ فَلَمَّا تَفَشَّأَ نَفْسُهُ نَحْبَتَهُ فَخَلَقْنَا لَهُ عَذَابًا أَخْرَ١٧ صَدَقَ اللَّهُ العَظِيم

المؤمنون: 12 - 14
Dedication

To the candle of my life:

(my father and mother)....

To my brothers and sisters

To my teachers

To my college and friends

I dedicate this work
Acknowledgment

My great ful thanks firstly to my God who guided me to the straight way in my life. Then many thanks and appreciations are extended to my mother, father and my husband for their support.

To my supervisor prof. Sana Eltahir for her valuable advice and endless efforts to make this work come into reality.
Abstract

This is an analytical descriptive study aimed to measure the complete blood count of Sudanese pregnant women who attended the Military Hospital in Omdurman, during January - April 2015.

The study included hundred healthy pregnant women. Two ml of venous blood were collected in EDTA anticoagulant for complete blood count which was measured by Sysmex(Kx_21N). Data were analyzed by independent test and one way ANOVA test using SPSS computer program version 11.5.

The pregnant women were divided into five age groups: less than 20 year, (20-25) year, (26-30) year, (31-35) year and more than 35 years. The distribution of the studied women according to the trimester was (48%), (38%) and (14%) for the third, second and first trimester respectively. History of abortion was found in (31%) of study group. Number of pregnancies were (49%), (38%) and (13%) for less than 3, (3-5) and more than 5 pregnancies respectively.

The result of the study showed that hemoglobin concentration, red blood cell count, hematocrit, mean cell volume, mean corpuscular hemoglobin and mean cell hemoglobin concentration were (11.9±1.00 g/dl), (4.2±0.41103 / μl), (35.0±2.74), (84.2±5.25fl), (28.6±2.25pg), (34.0±1.04%), white blood cell, lymphocyte and neutrophil were (7.6±1.85103 / μl), (26.3±7.06%) and (63.9±7.89%), the platelet count, (263.2±66.86 103 / μl.

There was significant difference between trimester and RBC, HCT, WBC, lymphocyte, neutrophil and platelet.

The CBC did not vary significantly with age, history of abortion and number of pregnancies. All the studied parameters were found to be within the normal range for pregnant women.

مستخلص البحث
هذه دراسة مقطعية وصفية تحليلية تهدف إلى قياس تعداد الدم الكامل للنساء الحوامل السودانيات

باستخدام السلاح الطبي بأمدرمان في الفترة ما بين يناير إلى أبريل 2015م.

وقد اشتملت الدراسة على مائة إمرأة من الحوامل. حيث اخذت 2ملم عينة دم وريدي ووضعت في حامض الخليط الثلاثي الأميني الثاني لتحديد الدم الكامل الذي يقبس بجهاز سيسمكس(\(Kx_{21N}\)),
وقد تمّ تحليل البيانات بواسطة برنامج حاسب الحزم الإحصائية للعلوم الاجتماعية نسخة 11.5 عبر
اختبار مستقل واختبار أنوفا ذات الاتجاه الواحد.

وقد تمّ تقسيم النساء الحوامل اللواتي خضعن للفحص في هذه الدراسة إلى خمس مجموعات: أقل من 20)، (20-25)، (25-30)، (30-35)، (أكثر من 35)، حيث وجد أن (48%) من النساء في المرحلة الثالثة من الحمل، بينما (38%) منهن في المرحلة الثانية من الحمل، وأنّ (14%) منهن في المرحلة الأولى من الحمل. فيما يتعلق بتاريخ الإجهاض فقد وجد أن (69%) من مجموعة النساء لم يجهضن
من قبل،بينما (31%) منهن كن قد أجهضن.

وقد توصلت الدراسة إلى عدة نتائج أهمها: أن متوسط مادة الهيموجلوبين في الدم ، و متوسط
هيموجلوبين كريات الدم الحمراء، و متوسط حجم كريات الدم الحمراء، والتركيز الوسطي للدم في
الكرية كانت كالاتي: (10.0±1.9)/(11.9±2.25)(g/dl)/(fl), (84.2±5.25), (28.6±2.25), (26.3±7.06)
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Chapter One

Introduction and literature review
Chapter One

1. Introduction and literature review

1.1 Introduction:

There are changes in the maternal circulatory system during pregnancy. Blood flow through the placenta and maternal cardiac output increase during pregnancy, about 625 milliliter of blood flow through the maternal circulation of the placenta each minute during the last month of pregnancy. (Guyton and Hall’s, 2011).

Pregnancy places extreme stresses on the hematological system and understanding of the physiological changes that results is obligatory in order to interpret any needs for therapeutic intervention. (Hoffbrand, 2006)

Normal human pregnancy lasts approximately 40 weeks, as measured from the first day of the last normal menstrual period “LNMP”. The anticipated date of an infant’s birth is commonly referred to as the expected date of confinement (EDC). When talking with patients, physicians customarily divide pregnancy into three times intervals called trimesters each of which is slightly longer than 13 weeks. By convention, the first trimester, 0 to 13 weeks, beings of the first day of the last menses. Rapid fetal growth occurs during the 13 to 26 weeks of second trimester. By the end of the second trimester, the fetus weighs approximately 700g and is 30cm long; many fetal organs begin to mature. The 26 to 40 weeks of the third trimester is the period in which fetal organs complete their prenatal maturation.
During this trimester, the growth rate decelerators, at the end of the third trimester, the fetus weighs approximately 3200g and is about 50cm long. Term is the interval from 37 to 42 weeks. Normal labor, rhythmic uterine contractions, and birth occur during this period. (Carl et al., 2008)

1.2 Literature review

1.2.1 Blood

1.2.1.1 Blood function

The function of blood is to serve the needs of the body tissues to transport nutrient to the body tissues, to transport waste products away, to transport hormones from one part of the body to another, and in general to maintain an appropriate environment in all the tissue fluids of the body for optimal survival and function of the cells. (Guyton and Hall, 2011)

1.2.1.2 Blood composition:

Blood consists of formed elements that are suspended and carried in fluid called plasma. The formed elements erythrocytes, leukocytes, and platelets. Function respectively in oxygen transport immune defense, and blood clotting. Plasma contains different types of protein and many water soluble molecules. The total blood volume in the average sized adult is about 5 liters consisting about 8% of the total body weight. Blood leaving the heart is referred to as arterial blood. Arterial blood with the exception of that going to the lungs is bright red because of the high concentration of oxyhemoglobin the combination of oxygen and hemoglobin in the red blood cells. Venous blood is blood returning to the heart. Except for the venous blood from the lungs, it contains less oxygen and is the therefore a darker red than the oxygen rich arterial blood. Blood is composed of a cellular protein, called formed elements and fluid protein, called plasma.
When a blood sample is centrifuged, the heavier formed elements are packed into the bottom of the tube leaving plasma at the top. The formed element constitute approximately 45% of the total blood volume a measurement called the hematocrit and the plasma account for remaining 55%. (FOX, 2006)

1.2.1.2.1 Plasma:
Plasma is a straw colored liquid consisting of water and dissolved solutes. The major solute of the plasma in terms of its concentration is Na+. In addition to Na+, plasma contains many other ions. As well as organic molecules such as metabolites, hormones, enzymes, antibodies, and other proteins. Plasma volume a number of regulatory mechanisms in the body maintains homeostasis of the plasma volume. If the body should lose water the remaining plasma become excessively concentrated. Its osmolality increase. (Fox, 2006)

1.2.2 Complete blood count (CBC):
The CBC is a basic screening test and is one of the most frequently order laboratory produce. The finding in the CBC gives valuable diagnostic information about the hematologic and other body system prognosis, response to treatment, and recovery. The CBC consists of a series of test that determine number, variety, percentage concentrations and quality of blood cells. (Fox, 2006)

1.2.2.1 Blood cells:
The formed elements of blood include three types of blood cells: erythrocytes or red blood cells, leukocytes or white blood cells and platelet or thrombocytes. (Fox, 2006)
1.2.2.2 Erythrocytes:

Erythrocytes are flattened, biconcave, discs about 7mm in diameter and 2.2mm thick. Their unique shape relates to their function of transporting oxygen. It provides an increased surface area through which gas can diffuse. Erythrocytes lack nuclei and mitochondrial (they obtain energy through anaerobic respiration). Partly because of these deficiencies erythrocytes have relatively short circulating life span of only about 120 days. Older erythrocytes are removed from the circulation by phagocytes cells in the liver, spleen, and bone marrow. Each erythrocyte contains approximately 280million hemoglobin molecules, which give blood its red color. Erythropoiesis is an extremely active process. It is estimated that about 2.5million erythrocytes are produced every second in order to replace those that are continuously destroyed by the spleen and liver. The production of red blood cells is stimulated by the hormone erythropoietin. (Fox, 2006)

1.2.2.3 Leukocytes:

Leukocytes differ from erythrocytes in several respects. Leukocytes contain nuclei and mitochondria and can move in an amoeboid fashion. Because of their amoeboid ability leukocytes can squeeze through pores in capillary walls and move to a site of infection, whereas erythrocytes usually remain confined within blood vessels. The movement of leukocytes through capillary walls is referred to as diapedesis or extravasations. White blood cells are almost invisible under the microscope unless they are stained. Therefore they are classified according to their staining properties. Those leukocytes that have granules in their cytoplasm are called granular leukocytes. Those without clearly visible granules are called a granular (non granular) leukocytes. (Fox, 2006)
1.2.2.3.1 Neutrophils:

Neutrophils are the most abundant type of leukocytes, accounting for 50 -70 % of the leukocytes in blood. Immature neutrophil have sausage shape nuclei and are called band cells. As the band cells mature their nuclei become lobulated, with two to five labs connected by thin strand. At this stage the neutrophil are also known as polymorphonuclear leukocytes (PMNS). (Fox, 2006)

1.2.2.3.2 Eosinophils:

These cells are similar to neutrophils, expect that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclears lobes. Eosinophil myelocytes can recognized but earlier stages are indistinguishable from neutrophil precursors. The blood transit time for eosinophils is longer than for neutrophils. They enter inflammatory exudates and have special role in allergic response, defence against parasites and removal of fibrin formed during inflammation. (Hoffbrand, 2006)

1.2.2.3.3 Basophils:

These are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granules which overlie the nucleus and contain heparin and histamine. In the tissue they become mast cells. They have immunoglobulin E (IgE) attachment sites and their degranulation is associated with histamine release. (Hoffbrand, 2006)

1.2.2.3.4 Lymphocytes:

Lymphocytes are the immunological competent cells that assist the phagocytes in defense of the body against infection and other foreign invasion.
The immune response depends upon two types of lymphocytes, B and T cells. (Hoffbrand, 2006)

1.2.2.3.4.1 B-lymphocyte:

B cells mature in the bone marrow and circulate in peripheral blood until they undergo recognition of antigen. The B-cell receptor is membrane bound immunoglobulin and after activation this is secreted as a free soluble immunoglobulin. At this point they mature to memory B cell or plasma cells. (Hoffbrand, 2006)

1.2.2.3.4.2 T-lymphocyte:

T cell develop from cell that migrated to the thymus where they differentiate into mature T cells during passage from the cortex to the medulla. During this process self-reactive T cells are deleted (negative selection) whereas T cells with some specificity for host human leucocytes antigen (HLA). (Hoffbrand, 2006)

1.2.2.3.5 Monocyte:

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

Platelets or thrombocytes are the smallest of the formed element and are actually fragment of large cells called megakaryocytes which are found in the bone marrow. (This is why the term formed element used instead of blood cells to describe erythrocytes, leukocytes and platelets). The fragments that enter the circulation as platelets lack number but like leukocytes are capable of amoeboid movement. The platelets count per cubic Nano meter of blood ranges from 130,000 to 400,000, but this count can vary greatly under different physiological condition platelets survive for about 5 to 9 days before being destroyed by the spleen and the liver. Platelets play an important role in the blood clotting. They constitute most the mass of the clot, and phospholipids in their cell membrane activate the clotting factors in plasma that result in thread of fibrin, which reinforce the platelets plugs. Platelets that attach together in blood clot release serotonin a chemical that stimulates constriction of blood vessels, thus reducing the flow of blood to the injured area, platelets also secrete growth factors which are important in maintaining the integrity of blood vessels. These regulators also may be involved in the development of atherosclerosis. (Fox, 2006)

1.2.2.5 Hemoglobin:

Hemoglobin molecule consist of four protein chain called globin's, each of which is bonded to one heme, a red pigmented that contains irons, the iron group of home is able to combine with oxygen in the lungs and release oxygen in the tissues. The heme iron recycled from senescent (old) red blood cells in the liver and spleen. This iron travel in the blood to bone marrow attach to protein carrier called transferrin. This recycled heme iron supplies most of the body need for iron. The balance of the requirement for iron, though relatively
small must be made up for in the diet. Dietary iron is absorbed mostly in the duodenum and transported from the intestine bound to transferrin in blood. Transferrin is taken out of the blood when the transferrin molecule binds to receptor proteins on the plasma membranes of cells, triggering endocytosis. (Fox, 2006)

Most automated counters measure Hb by a modification of the manual hemoglobin cyanide (HiCN) method. Modifications include alterations in the concentration of regents and in the temperature and pH of the reaction. A non-ionic detergent is included to ensure rapid cell lysis and to reduce turbidity due to the cell membranes and plasma lipids. Measurements of absorbance are made at a set time interval after mixing of blood and the active regents but before the reaction are completed. In some HiCN has been replaced with a method using a non-toxic chemical, sodium lauryl sulphate, in order to reduce possible to environmental hazard from disposal sysmex (Toa) instrument, of large volume of cyanide-containing waste. This has been found to be a reliable routine method with estimations of Hb being generally equivalent to those produced by the HiCN method. (Dacie and lewis, 1995)

**1.2.2.6 Hematocrit (HCT):**

Also known as packed cell volume. The word hematocrit means to separate (which underscores the mechanism of the test because the plasma and blood cells are separated by centrifugation). The HCT test is part of CBC. This test indirectly measures the RBC’s mass. The result are expressed as the percentage by volume of the packed RBC’s in whole blood (PCV). It is an important measurement in the determination of anemia or polycythemia. (fischbach, 2009)
1.2.2.7 Red blood cells indices:

The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV). The mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular hemoglobin (MCH).

Table(1.1) value in non-pregnant women: (fishchbach, 2009)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>82-98FL</td>
</tr>
<tr>
<td>MCHC</td>
<td>32-36%</td>
</tr>
<tr>
<td>MCH</td>
<td>26-34Pg</td>
</tr>
<tr>
<td>HCT</td>
<td>36% - 48%</td>
</tr>
<tr>
<td>RDW</td>
<td>11.5 – 14.5%</td>
</tr>
</tbody>
</table>
1.2.2.8 Red blood cell count (RBC):

Red cells and other blood cells may be counted electronically in a system based on aperture impedance or light-scattering technology. Cells are counted as they pass in a stream through an aperture. Large numbers of cells can be counted so that the precision of an electronic red cell count is much better than that of a manual count and the count is available in a fraction of the time. As a consequence, electronic counts have rendered the RBC and the red cells indices derived from it (the MCV and the MCH) of much more clinical relevance than when only a slow and imprecise manual RBC was available. (Dacie and Lewis, 1995).

1.2.2.9 The distribution of red cell volume-red cell distribution (RDW):

This automated method of measurement is helpful in the investigation of some hematological disorder and in monitoring response to therapy. The RDW is essentially an indicator of the degree of anisocytosis (abnormal variation in size of RBC’s) normal RBC’s have slight degree of variation. The RWD also can be helpful in distinguishing anemia of chronic disease (low-normal MCV, normal RWD) from early iron deficiency anemia (low-normal MCV, elevated RWD). Reference value of RDW is 11.5 – 14.5%. (Fishbach and Dunning, 2009).

Automated instrument produce volume distribution histograms which allow the presence of more than one population of cells to be appreciated. Instrument may also assess the percentage of cells falling above below given MCV thresholds and “flag” the presence of an increased number of microcytes or macrocytes. Such measurement may indicate the presence of a small but significant increase in the percentage of either microcytes or macrocytes before there has been any
change in the MCV. Most instrument also produce a quantitative measurement of the variation in cell volume, an equivalent of the microscopic assessment of the degree of anisocytosis. This new parameter has been named the “red cell distribution width” (RDW). The RDW is derived from pulse height analysis and can be expressed either as the standard deviation (in fl) or as the coefficient of variation (%) of the measurement of the red cell volume. Current coulter and technicon instrument express the RDW as the SD, and sysmex(Toa) instrument express it as either the SD or the CV. Widely different reference ranges have been reported for the RDW with the CV varying between 7.4 and 13.4%. It is therefore important for laboratories to determine their own reference range. The RDW expressed as the CV has been found of some value in distinguishing between iron deficiency (RDW usually increased) and thalassaemia trait (RDW usually normal) and between meagloblastic anemia (RDW often increased) and other cause of the marcocytosis (RDW more often normal). (Dacie and lewis, 1995).

1.2.2.10 Total white blood cell count (WBC):

The total white cell count is determined in whole blood in which red cells have been lysed. The lytic agent required to destroy the red cells and reduce the red cell stroma to residue which causes no detectable response in the counting system without affecting leucocytes in such a manner that the ability of the system to count them altered. Various manufacturers recommend specific reagents and for multi-channel instrument which also perform an automated differential count use of the recommended reagent is essential. For simple single-channel impedance counter the following fluid is satisfactory. (Dacie and lewis, 1995)
1.2.2.11 Automated differential count:
Automated differential counters which are now available generally use flow cytometry. Incorporated into a full blood counter rather than being stand-alone differential counters. Differential counters based on pattern recognition in stained blood films were initially preferred by many haematologist but they were relatively slow, and since they could count only small number of cells in reasonable time the precision of the automated count was no better that of a manual count. (Dacie and lewis, 1995).

1.2.2.12 Manual count:
Better and more reliable results are obtained with new methylene blue than with brilliant cresyl blue. New methylene blue stains the reticulofilamentous material more deeply and more uniformly than does brilliant cresyl blue, which varies from sample to sample in its staining ability. Purified azure B is a satisfactory substitute for new methylene blue; it has the advantage that the dye does not precipitate and it is available in pure form. It is used in the same concentration and the staining procedure is the same as with new methylene blue. (Dacie and lewis, 1995)

1.2.3 Pregnancy:
Pregnancy is the presence of a developing offspring in the uterus. It results from the union of the genetic packages of an egg cell and a sperm cell an event called fertilization. (David etal., 2004).
The zygote divide, becoming a morula. After 50 to 60 cells present, the morula develops cavity, the primitive yolk sac, and thus becomes blastocysts which implants into the uterine wall about 5 days after fertilization. The cell on the exterior wall of the blastocyst, trophoblast, synergistically invade the uterine
endometrium and develop into chorionic villi, creating the placenta. A cavity called the amnion forms and enlarge with the accumulated of amniotic fluid an embryo undergoes rapid cell division, differentiation, and growth form combinations of ectoderm, mesoderm, and endoderm, organs begin to form, a process called organogenesis. At 10 weeks, an embryo has developed most major structures and is non-referred to as a fetus. At 13 weeks, the fetus weighs approximately to 13g and is 8cm long. Rapid fetal growth occurs during the 13 to 26 weeks of second trimester. By the end of the second trimester, the fetus weighs approximately 700g and is 30cm long; many fetal organs begin to mature. The 26 to 40 weeks of the third trimester is the period in which fetal organs complete their prenatal maturation. During this trimester, the growth rate decelerators, at the end of the third trimester, the fetus weighs approximately 3200g and is about 50cm long. Term is the interval from 37 to 42 weeks. Normal labor, rhythmic uterine contractions, and birth occur during this period. (Carl et al., 2008)

1.2.3.1 Physiological changes during pregnancy:

1.2.3.1.1 Hormonal changes:

During a typical reproductive cycle, the corpus luteum degenerates about two weeks after ovulation. Consequently, concentrations of estrogens and progesterone decline rapidly, the uterine lining is no longer maintained and the endometrium slough off as menstrual flow if this occurs following implantation, the embryos is lost (spontaneously aborted). A hormone called human chorionic gonadotropin (HCG) normally helps prevent spontaneous abortion. A layer of cells, called atrophoblast, that secretes HCG and later helps form the placenta, surrounds the developing embryo. (David et al., 2004)
1.2.3.1.2 Hematological changes during pregnancy:

Maternal blood volume increases during pregnancy by an average 45%. Plasma increases more rapidly than red blood cells masses. Therefore, despite augmented erythropoiesis hemoglobin concentration, erythrocytes count and hematocrit decrease during normal pregnancy. Hemoglobin concentration at term average 12.6g/dl, compared with 13.3g/dl for the non-pregnant state. The concentration of the several blood coagulation factors are increased during pregnancy. (Carl etal., 2008)

Physiological changes in pregnancy and puerperium are principally influenced by changes in the hormonal milieu. Many hematological changes also, occurring during these periods are physiological and are of inconsequential concern to the hematologist (Chandra Setal.,2012).

1.2.3.1.2.1 Red Blood Cells

During pregnancy, the total blood volume increases by about 1.5 liters, mainly to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery. Of this, around one liter of blood is contained within the uterus and maternal blood spaces of the placenta. Increase in blood volume is, therefore, more marked in multiple pregnancies and in iron deficient states. Expansion of plasma volume occurs by 10–15 % at 6–12 weeks of gestation. During pregnancy, plasma renin activity tends to increase and atrial natriuretic peptide levels tend to reduce, though slightly. This suggests that, in pregnant state, the elevation in plasma volume is in response to an under filled vascular system resulting from systemic vasodilatation and increase in vascular capacitance, rather than actual blood volume expansion, which would produce the opposite hormonal profile instead (i.e., low plasma renin and elevated atrial
Red cell mass (driven by an increase in maternal erythropoietin production) also increases, but relatively less, compared with the increase in plasma volume, the net result being a dip in hemoglobin concentration. Thus, there is dilutional anemia. The drop in hemoglobin is typically by 1–2 g/dL by the late second trimester and stabilizes thereafter in the third trimester, when there is a reduction in maternal plasma volume (owing to an increase in levels of atrial natriuretic peptide). Women who take iron supplements have less pronounced changes in hemoglobin, as they increase their red cell mass in a more proportionate manner than those not on hematinic supplements (Chandra et al., 2012).

The red blood cell indices change little in pregnancy. However, there is a small increase in mean corpuscular volume (MCV), of an average of 4 fl in an iron-replete woman, which reaches a maximum at 30–35 weeks gestation and does not suggest any deficiency of vitamins B12 and folate. Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to a higher proportion of young RBCs which are larger in size). However, MCV does not change significantly during pregnancy and a hemoglobin concentration <9.5 g/dL in association with a mean corpuscular volume <84 fl probably indicates co-existent iron deficiency or some other pathology (Chandra et al., 2012).

Post pregnancy, plasma volume decreases as a result of diuresis, and the blood volume returns to non-pregnant values. Hemoglobin and hematocrit increase consequently. Plasma volume increases again two to five days later, possibly because of a rise in aldosterone secretion. Later, it again decreases. Significant elevation has been documented between measurements of hemoglobin taken at 6–8 weeks postpartum and those taken at 4–6 months postpartum, indicating
that it takes at least 4–6 months post pregnancy, to restore the physiological dip in hemoglobin to the non-pregnant values (Chandra et al., 2012).

**1.2.3.1.2.2 White Blood Cells**

White blood cell count is increased in pregnancy with the lower limit of the reference range being typically 6,000/cumm. Leukocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state. Neutrophils are the major type of leucocytes on differential counts. This is likely due to impaired neutrophilic apoptosis in pregnancy. The neutrophil cytoplasm shows toxic granulation. Neutrophil chemo taxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female. There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. Immature forms as myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance. They simply indicate adequate bone marrow response to an increased drive for erythropoiesis occurring during pregnancy (Chandra et al., 2012).

Lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester. There is an absolute monocytosis during pregnancy, especially in the first trimester, but decreases as gestation advances. Monocytes help in preventing fetal allograft rejection by infiltrating the decidual tissue (7th–20th week of gestation) possibly, through PGE2 mediated immunosuppression. The monocyte to lymphocyte ratio is markedly increased in pregnancy. Eosinophil and basophil counts, however, do not change significantly during pregnancy (Chandra et al., 2012).

The stress of delivery may itself lead to brisk leukocytosis. Few hours after delivery, healthy women have been documented as having a WBC count
varying from 9,000 to 25,000/cumm. By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (Chandra Setal., 2012).

1.2.3.1.2.3 Platelets

Large cross-sectional studies done in pregnancy of healthy women (specifically excluding any with hypertension) have shown that the platelet count does decrease during pregnancy, particularly in the third trimester. This is termed as “gestational thrombocytopenia.” It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance. Gestational thrombocytopenia does not have complications related to thrombocytopenia and babies do not have severe thrombocytopenia (platelet count ≤20,000/cumm). It has hence been recommended that the lower limit of platelet count in late pregnancy should be considered as 1.15 lac/cumm. The platelet volume distribution width increases significantly and continuously as gestation advances, for reasons cited before. Thus, with advancing gestation, the mean platelet volume becomes an insensitive measure of the platelet size. Post-delivery platelet count increases in reaction to and as a compensation for increased platelet consumption during the process of delivery (Chandra Setal., 2012).

1.2.3.2 Changes in the maternal circulatory system during pregnancy:

Blood flow through the placenta and maternal cardiac output increase during pregnancy, about 625 milliliter of blood flow through the maternal circulation of the placenta each minute during the last month of pregnancy. This plus the general increase the mother’s cardiac output to 30 to 40 percent above normal by the 27 week of pregnancy, for reason unexplained, the cardiac output falls to
only a little above normal during the last weeks of pregnancy, despite the high uterine blood flow. Maternal blood volume increase during pregnancy. The maternal blood volume shortly before term is about 30% above normal. This increase occurs mainly during the latter half of pregnancy. The cause of the increased volume is likely due to the least in part to aldosterone and estrogen, which are greatly increased in pregnancy and to increased fluid retention by the kidneys, also the bone marrow increasingly active and produce extra red blood cells to go with the excess fluid volume. Therefore, at the time of birth of baby, the mother has about 1 to 2 liters of extra blood in her circulatory system; only about fourth of this amount is normally lost through bleeding during delivery of the baby there by allowing a considerable safety factor for mothers. (Guyton and Hall’s, 201).
1.3 Rationale:

There are many changes occurs in the maternal circulation during pregnancy, and the pregnant women needs to be up to date with the changes that occur during pregnancy. Pregnancy places extreme stresses on the hematological system and understanding of the physiological changes that results is obligatory in order to interpret any needs for therapeutic intervention. Maternal blood volume increases during pregnancy by an average 45%. Plasma increases more rapidly than red blood cells masses. Therefore, despite augmented erythropoiesis hemoglobin concentration, erythrocytes count and hematocrit decrease during normal pregnancy. Blood flow through the placenta and maternal cardiac output increase during pregnancy, about 625 milliliter of blood flow through the maternal circulation of the placenta each minute during the last month of pregnancy. A Complete Blood Count assesses all components of the blood (red blood cells, white blood cells and platelets which are involved in clotting.) An abnormally high or low count could indicate the presence of various diseases.

In pregnancy, haemoglobin (red blood cell count) levels will be checked to make sure blood can carry enough iron and oxygen. Because of the normal changes in pregnancy, it is very common for the haemoglobin to go down. Women experience a 50% increase in their blood volume during pregnancy but only a 30% increase in the number of red blood cells. As a result, most women will develop what is called physiologic anemia. Anemia can be especially troubling in pregnancy because a woman needs enough red blood cells to carry oxygen around her body and to her baby; if the haemoglobin is low, the pregnant women feel tired, weak and dizzy that is above and beyond normal pregnancy symptoms.
Depending on the haemoglobin levels, The health care provider may suggest taking an iron supplement such as ferrous gluconate; 300 mg once a day with a glass of orange juice (Vitamin C helps absorb the iron) is usually recommended. Foods high in iron will also help with haemoglobin levels, these include: lean red meats, egg yolks, liver and spleen all of which should be part of a balanced healthy diet.
1.4 Objectives:

1.4.1 General objective:
To measure complete blood count among pregnant women attended Military Hospital in Omdurman.

1.4.2 Specific objective:
- To measure Hb, RBC, HCT, MCV, MCH, MCHC, WBC, differential leukocytes and platelets in pregnant women
- To assess the effect of the trimester on complete blood count.
- To assess the effect of age on complete blood count.
- To assess the effect of history of abortion on complete blood count.
- To assess the effect of number of pregnancies on complete blood count.
CHAPTER TWO

Materials and Methods
Chapter Two

2. Materials and Methods

2.1 Study design:
This is a descriptive study conducted in Sudanese pregnant women.

2.2 Study Area and duration:
Sample was collected from pregnant women who attended Military Hospital in Omdurman in the period from January to April 2015.

2.3 Study population:
Hundred Sudanese pregnant women attended Military Hospital.

2.4 Inclusion criteria:
Pregnant women attended the Military Hospital with normal pregnancy, no medical complication or history of drug in take were included in the study.

2.5 Exclusion criteria:
Pregnant women with complications of pregnancy, history of medical consideration, or drug in take that affect the result were excluded from the study.

2.6 Data collection:
Data was collected by constructed questionnaire about age, trimester, history of abortion and number of pregnancies.
2.7 Sample Collection:

Two ml of venous blood was collected by disposable syringe after cleaning the skin by cotton containing alcohol, then withdrawn in EDTA container and mixed with the anticoagulant to avoid clot formation.

2.8 Materials:

Instrument and equipment:

1) EDTA container
2) Disposable syringes
3) Tourniquet
4) Cotton wool
5) Automatic pipette
6) Blue and yellow tips
7) Microscope
8) Slides
9) Sysmex Kx_21N

2.9 Reagents:

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

2.10 Methods:

2.10.1 Complete blood count:

Complete blood count (HB, RBcs, WBC, MCV, MCH, MCHC, HCT, platelet indices) was tested by automated method (Sysmex).
2.10.2 Principle:

Sysmex (kx-21N) is a hematology automated analyzer, used to quickly perform full blood counts and reticulocyte counts, it is made by the Sysmex Corporation. Blood is sampled and diluted, and moves through a tube thin enough that cells pass by one at a time. Because not everything about the cells can be measured at the same time, blood is separated into a number of different channels. As the cells pass through apertures the signals are transmitted in sequence to analog circuit and then to particle size distribution analysis circuits for conversion to cumulative cell size distribution data, particle size distribution curves constructed, and the auto discrimination level is then set by the microprocessor for each population. This floating threshold allows for discrimination of all populations, the cell count includes the pulses between the lower and the upper auto discrimination level.

2.11 Procedure:

The reagent for operation were checked, then the power switch was turned on auto rise and background check were automatically performed, then three levels of control (low count, normal and high) were applied after selection whole blood mode of analysis sample numbers were introduced by pressing sample number keys then enter key was pressed, after that sample was mixed carefully the tab bring in close contact with sample probe and the start key was pressed, the required volume of blood were aspirated, then the LCD screen display analyzing the tube was removed, result was displayed in the screen and was printed out. (Sysmex America Inc, 2003).
2.12 Quality control of sysmex:

hematology analyzers provide quick and accurate results in most situations. However, false results related either to platelets or other parameters from complete blood count may be observed in several instances, false low white blood cell (WBC) counts may be observed because of agglutination in the presence of ethylenediamine tetra-acetic acid (EDTA).

2.13 Ethical considerations:

Ethical clearance was obtained from Military Hospital and Research council of the collage of medical laboratory sciences and samples were collected. Consents were taken from all the participations after they had been informed about the procedure of blood collection and aims of study. They were used that data will be kept confidentially and will not be used for any other purpose than this study.

2.14 Data analysis:

The effect of age, trimester, history of abortion and number of pregnancies was tested by using SPSS version 11.5.
Chapter Three
Results
Chapter Three

3. Results

Complete blood count of pregnant women, showed that according to age the pregnant women were divided into five groups, the most frequent age group was (20-25) years (42%) while the least frequent group was than 35 years (7%) (Figure 3.1). According to the trimester (48%) of women were in the third trimester, (38%) were in the second trimester, (14%) of the study groups were in the first trimester (figure 3.2). According to the number of pregnancies (49%) of the pregnant women have less than 3 pregnancies while pregnant women with more than 5 pregnancies represented the least number (13%) (Figure 3.4). According to history of abortion only (31%) was found to have a history of abortion (Figure 3.3).

Table (3.2) shows that HCT (35.2±2.8%) of pregnant women of age group ranged between (20-25) years increased insignificantly (p.value 0.938) compared with (less than 20), (26-30), (31-35), (more than 35). Platelets (237.1 ± 62.9× 10^3 / µl) of age group more than 35 years decreased insignificantly (p.value 0.306). RBCS (4.02 ± 0.33 × 10^3 / µl), HCT (34.03±2.18%) of pregnant women decreased significantly (p.value 0.015, 0.025) in the second trimester while Hb (11.69±0.79 g/dl) decreased insignificantly (p.value 0.088) compared to the first and third trimester (table 3.3).

The results showed that platelet (246.6±62.63× 10^3 / µl) and lymphocyte (23.93±5.61%) of pregnant women in the third trimester decreased significantly (p.value 0.053, 0.000) compared to first and second trimester (table 3.3). WBC (7.71±1.14× 10^3 / µl) and neutrophils (66.13±6.18%) increased...
significantly (p.value 0.004, 0.000) in the third trimester compare to first and second trimester. while MCV, MCH, MCHC (83.12±5.37fl), (27.98±1.81pg) ,(33.84±1.23%) decreased insignificantly in first trimester compare to second and third trimester.

Hb (11.72±1.09g/dl), HCT (34.92±3.22%) and platelet (240.62±70.40×10^3 / μl) of pregnant women with number of pregnancies more than 5 decreased insignificantly (p.value 0.567, 0.485, 0.113) than of the (less than 3) and (3-5), but MCV (85.6±4.96fl), MCH (29.21±1.76pg), MCHC (34.27±0.85%) increased insignificantly (p.value 0.368, 0.402, 0.425), WBC (7.13±1.68×10^3 / μl), neutrophil (61.73±8.13%) decreased insignificantly in pregnant women with number of pregnancies (3-5) than (less than 3) and (more than 5), Mix (10.11±1.92%) and lymphocyte (28.16±7.46%) increased insignificantly.

Hb, HCT, MCV, MCH, MCHC (11.85±1.06g/dl), (34.90±2.79%), (83.88±5.04fl), (28.52±2.24pg), (33.96±1.10%) decreased insignificantly, Platelet increased significantly of pregnant women with no history of abortion compare to history of abortion (11.99±0.87g/dl), (HCT, MCV, MCH, MCHC decreased insignificantly in the group with no history of abortion.
Figure (3.1): Distribution of study population according to age

Figure (3.2): Distribution of study population according to trimester of pregnancy
Figure (3.3): Distribution of study population according to history of abortion

Figure (3.4): Distribution of study population according to number of pregnancies
Table (3.1): The means value of Complete Blood Count of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>Mean±STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC $\times 10^3$ $/ \mu l$</td>
<td>Case</td>
<td>4.2±0.41</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>Case</td>
<td>11.9±1.00</td>
</tr>
<tr>
<td>HCT%</td>
<td>Case</td>
<td>35.0±2.74</td>
</tr>
<tr>
<td>MCVfl</td>
<td>Case</td>
<td>84.2±5.25</td>
</tr>
<tr>
<td>MCHpg</td>
<td>Case</td>
<td>28.6±2.25</td>
</tr>
<tr>
<td>MCHC%l</td>
<td>Case</td>
<td>34.0±1.04</td>
</tr>
<tr>
<td>PLT $\times 10^3$ $/ \mu l$</td>
<td>Case</td>
<td>263.2±66.86</td>
</tr>
<tr>
<td>WBC $\times 10^3$ $/ \mu l$</td>
<td>Case</td>
<td>7.6±1.85</td>
</tr>
<tr>
<td>LYM%</td>
<td>Case</td>
<td>26.3±7.06</td>
</tr>
<tr>
<td>MIX%</td>
<td>Case</td>
<td>9.8±2.28</td>
</tr>
<tr>
<td>NEUTO%</td>
<td>Case</td>
<td>63.9±7.89</td>
</tr>
</tbody>
</table>
Table (3.2): The Effect of the age on Complete Blood Count of study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age group(years)</th>
<th>Mean± STD.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC×10^3/μl</td>
<td>Less than 20</td>
<td>4.23 ± 0.34</td>
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<td>249.97±57.91</td>
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<td>31 – 35</td>
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<td>20 – 25</td>
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<td>20 – 25</td>
<td>26 – 30</td>
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Table (3.3): The Effect of the trimester on Complete Blood Count of study

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<th>P-value</th>
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<td>4.29±0.34a</td>
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<td></td>
<td>Second</td>
<td>4.02±0.33b</td>
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<tr>
<td></td>
<td>Third</td>
<td>4.25±0.45a</td>
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<td><strong>Hbg/dl</strong></td>
<td>First</td>
<td>11.99±0.79a</td>
<td>0.088</td>
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<td></td>
<td>Second</td>
<td>11.62±0.79a</td>
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<td></td>
<td>Third</td>
<td>12.09±1.16a</td>
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<td><strong>HCT%</strong></td>
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<td>35.48±2.72a</td>
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<td>Second</td>
<td>34.03±2.18b</td>
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<td>Third</td>
<td>35.58±2.98a</td>
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<td><strong>MCVfl</strong></td>
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<td>83.12±5.37a</td>
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<td>Second</td>
<td>84.70±4.67a</td>
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<td>Third</td>
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<td>Third</td>
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<td>55.91±8.65b</td>
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<td>Second</td>
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<td>Third</td>
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Mean with in the column followed by different superscript are significantly difference at p <0.05
Table (3.4) : The Effect of the history of abortion on Complete Blood Count of study population

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Table (3.5): The Effect of the number of pregnancies on Complete Blood Count of study population

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<td>4.04±0.54</td>
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<td>3 through 5</td>
<td>35.30±2.37</td>
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<td>84.53±5.11</td>
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Chapter Four
Discussion, Conclusion and Recommendations
Chapter Four
4. Discussion, Conclusion and Recommendations

4.1 Discussion:

The results of Hb (11.62±0.79 g/dl) level in pregnant women were decreased insignificantly (p.value 0.088) in second trimester compared with the other trimester, while HCT (34.03±2.18%) decreased significantly (p.value 0.025) while HCT (34.03±2.18%) decreased significantly (p.value 0.025), this agree with (Sara et al., 2012) who found the mean Hb concentration values declined in the first and second trimesters and reached (11.6±1.2 g/dL) at 24 weeks gestation in the Caucasian population. In these women the mean HB values declined in the first through second trimesters and slowly rose during the third trimester. The mean HCT values in the non-Caucasian pregnant women were significantly lower than the mean values in Caucasian pregnant women starting at week 30 and continuing through week 39 (P at least <0.05 at all-time points).

The White blood cell (6.14±2.06x10³/µl) decreased significantly in the first trimester (p.value 0.004), while the platelet decreased significantly in third trimester (246.69±62.63x10³/µl) (p.value 0.053), this agree with Akinbami et al., (2013) who found a statistically significant relationship between packed cell volume and white blood cell count with gestational age (P = 0.010 and 0.001, respectively). However, there was no statistically significant association between platelet count and gestational age (P = 0.296).
Rania (2012) observed that Hb concentration of pregnant women did not vary significantly from that of non pregnant women.

Rawia (2013) reported that values of the measured hematological parameters agree with normal value.
4.2 Conclusion:

- After completion of the study the result concluded that: RBCs, LYM, MCV and HCT significantly decreased. Hb and platelet decreased insignificantly but MCHC, MIX and Neutrophil significantly increased, MCH and WBCS insignificantly.
- According to number of pregnancies Hb, RBCs, HCT, MIX and platelet decreased insignificantly in pregnant women with number of pregnancies more than 5, while WBCs, MCV, MCH and MCHC increased insignificantly.
- According to trimester Hb decreased insignificantly in second trimester, HCT increased significantly in second trimester, MCH, MCV and MCHC decreased insignificantly in first trimester, LYM and platelet decreased significantly in third trimester, but WBCs and Neutrophil increased significantly in third trimester.
- According to history of abortion Hb, HCT, MCV, MCH, MCHC, MIX and WBCs decreased insignificantly in pregnant women with no history of abortion, LYM and Neutrophil increased significantly.
- According to age RBCs, Neutrophil and HCT increased insignificantly in pregnant women aged (20-25) years, MCHC and WBCs decreased insignificantly compared to other group, MCH, Mix and MCV decreased insignificantly in pregnant women with age less than 20 years, but Hb increased insignificantly.
- The study revealed that the age, history of abortion and number of pregnancies did not affect the complete blood count, also there significant affect of these results and trimester.
4.3 Recommendation:
This study recommended the following:

1- Another study should be conducted with a large sample size with additional other hematological parameter, iron profile and coagulation parameters.

2- Pregnant women should take balance food and iron supplement.

3- Iron status should be regularly assessed during pregnancy.

4- CBC should be regularly checked during pregnancy.
References
Reference:


5. Elsaid R,(2013), measurement of complete blood count among Sudanese pregnant women in Khartoum teaching hospital, , M.S.c thesis, sudan university of science and technology


Appendices
Name : ..............................................................................................................

No: ......................................................................................................................

Date: .....................................................................................................................

Age : .......................................................................................................................

Trimester of Pregnancy :

Abortion:

Follow up :

Others :

Test and result

HB: HCT: TOTAL RBC:
TOTAL WBC: platelet count: MCV:
MCH: MCHC: LYM:
MIX: NEUT: RDWC:
RDWS: PDW: MPV:
PLCR: