

Chapter One

Introduction and Literature review

1.1 Introduction:

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring develops inside a woman. Multiple pregnancies involve more than one offspring such as twins. Childbirth typically occurs around 40 weeks from the last menstrual period [LMP] (Abman and Steven, 2011).

Normal pregnancy is characterized by profound changes in almost every organ and system to accommodate the demands of the fetoplacental unit.

Pregnancy is influenced by many factors, some of which include culture, environment, socioeconomic status, and access to medical care (Yip, 2000).

Pregnancy is typically divided into three trimesters. The first trimester is from week one through 12 weeks and includes conception (Abman and Steven, 2011). It carries the highest risk of miscarriage (natural death of embryo or fetus). The second trimester is from week 13 through 28 weeks. Around the middle of the second trimester, movement of the fetus may be felt. At 28 weeks, more than 90% of babies can survive outside of the uterus if provided high-quality medical care. The third trimester is from 29 weeks through 40 weeks (Lippincott and Wilkin, 2012).

During pregnancy, the woman undergoes many physiological changes, which are entirely normal, including cardiovascular, hematologic, metabolic, renal and respiratory changes that become very important in the event of complications. The body must change its physiological and homeostatic mechanisms in pregnancy. Levels of progesterone and oestrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle (Ornoy and Ergaz, 2010).

There are many hematological changes during pregnancy such as increase in plasma volume by 50% and the red blood cell volume increased 20-30%. Consequently, the hematocrit decrease due to the dilution. White blood cell count increase and may peak at over 20 mg/mL in stressful conditions. Conversely, there is a decrease in platelet concentration to minimal normal values of 100-150mil/mL (Guyton and Hall, 2005).

1.2 Literature review:

1.2.1 Blood:

Blood is a combination of plasma and cells that circulate through the entire body. It is a specialized bodily fluid that supplies essential substance around the body, such as sugars, oxygen and hormones (Higgins, 2015).

1.2.1.1 Function of blood:

Blood has three main functions: transport, production and regulation.

Transport: Blood transport gases, waste, hormones and heat to skin so as to help regulate temperature.

Protection: Blood has several roles in inflammation such as white blood cell can destroy invading microorganisms, antibodies and other proteins destroy pathogenic substances and platelet factors initiate blood clotting.

Regulation: Blood helps regulate PH by interacting with acids and bases (Saladin, 2008).

1.2.2 Haemopoiesis:

It is the formation of blood cellular components. Which are derived from haematopoietic stem cells (Birbra and Frenette, 2016).

1.2.2.1 Site of Haemopoiesis:

Production of blood cells commences in the yolk sac called blood islands of the embryo and then shifts to the liver and to a lesser extent to the spleen. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism (Birbrair and Frenette, 2016)

1.2.2.2 Hematopoietic growth factors:

A glycoprotein hormone that regulates proliferation and differentiation of hematopoietic progenitor cells and function of mature blood cells. HGFs are chemicals, generally cytokines and interleukins that interact with

developing immature marrow cells and lead to greater numbers of red cells, white cells or platelets combinations of these (Rizzo *et al.*,2010)

1.2.3 Erythropoiesis:

Erythropoiesis is the process which produces red blood cells (erythrocyte).It is stimulated by decreased O₂in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin (Palis and Segel,2000).

1.2.3.1Stage of erythropoiesis:

Pro-erythroblast: 14-20um, nucleus with clumped chromatin, nucleoli have disappeared (Farlex ,2012) .

Basophile erythroblast: diameter of 12-16um ,nucleated precurer , cytoplasm is basophilic , nucleus is large with clumped chromatin (Farlex ,2012).

Polychromatic erythroblast: is a round cell between 12-14um in diameter.

Orthochromatic erythroblast: immature erythrocyte, before nuclear loss (Stacey and Blachford ,2000).

Reticulocytes have the same biconcave discoid shape as mature red cells, although they have a slightly greater volume and diameter than the latter (Davis and Bigelow, 2000)

Erythrocyte (mature red cell) cytoplasm pink colour, have lifespan 120 days diameter 6-8Mm.

1.2.3.2Erythropoietin (EPO):

Its heat stable glycoprotein with a molecular weight of about 34 KDa produced mainly in the kidney. Only a small quantity is demonstrable in normal plasma or urine (Dacies and Lewis 2001).

1.2.4Red blood cell count:

Red blood cell counts approximates the number of circulating red blood cell and used to help in diagnosis of anemia, polythythemia,

other myeloproliferation disorders and calculation of RBCs indices (Estridge *et al.*,2000).

High red blood cell count may be due to low oxygen levels, kidney disease as well as dehydration, smoking, polycythemia .Low red blood cell count causes by anemia include trauma, acute or chronic bleeding, bone marrow disorder (Schrier,2015).

$$\text{RBCs count} = \frac{\text{sum} \times \text{dilution factor}}{\text{Area} \times \text{depth}}$$

Normal range of pregnant women in first trimester $(3.42-4.55) \times 10^{12} / \text{L}$.In second trimester $(2.81-4.49) \times 10^{12} / \text{L}$.In third trimester $(2.71-4.43) \times 10^{12} / \text{L}$ (Greer and Cunningham,2009).

1.2.5 Packed cell volume (PCV):

Packed red blood cells are red blood cells that have been collected, processed and stored in bags as blood product unit available for blood transfusion (Carson *et al.*, 2016).

When hematocrit count is high that indicate to others causes such as:capillary leak syndrome, polycythemia vera, myeloproliferation disorder and dehydration. Anemia is the most common cause of low PCV. Normal range in males 40-45%.and females 38-42% (Johon,2009).

Normal value of pregnant women in first trimester (32.7 ± 6.8) .In second trimester (29.7 ± 5.2) . In third trimester $(33.0 \pm 3.8.0)$ (Akinbami *et al.*,2013).

1.2.6 Mean corpuscular volume (MCV):

It is measure of the average volume of a red blood corpuscle (or red blood cell).The mean corpuscular volume is a part of a standard complete blood count (Carson *et al.*,2016).

$$\text{Mean cell volume (MCV)} = \frac{\text{PCV} \times 10 \text{ FL}}{\text{RBCs}}$$

High level of MCV can causes due to liver disease and alcohol abuse as well as myelofibrosis and reticulocytosis.

Low level of MCV can causes due to lead poisoning, long-lasting kidney failure, long-term decrease of iron in the body and anemia .(Drew,2018)

Normal rang in pregnant women by μ^3 in first trimester (85-97.8). In second trimester (85.5-99.4). In third trimester (82.4-100.4) (Fischbach and Dunning,2009).

1.2.7 Mean corpuscular hemoglobin (MCH):

It is measure of the concentration of heamoglobin in given volume of packed red blood cells . Reference range of blood tests are 32 to 36 g / dL(Rifkind and Cohen,2002).

Different types of anemia can cause low MCH level. For e.g microcytic anemia occurs when the blood cell are too small and cannot take in as much hemoglobin as they should .high MCH scores are commonly a sign of macrocytic anemia,liver diseases and drinking alcohol regularly (Johnson and Graham,2011).

Mean cell haemoglobin:

$$\text{MCH} = \frac{\text{Hb(g/dl)} \times 10 \text{ pg}}{\text{RBCs in million.}}$$

1.2.8 Mean corpuscular hemoglobin concentration (MCHC):

It is measure of the concentration of heamoglobin in a given volume of packed red blood cells. Reference ranges for blood tests are 32 to 36 g/ dL (Lippincott and Williams,2007).

Mean cell hemoglobin concentrate (MCHC) = $\frac{\text{Hb (g/dl)} \times 100 \%}{\text{pcv}}$.

pcv

hemolysis can causes increase of MCHC. also MCHC level can be too low due to blood loss over time, too little iron in the body, or hypochromic anemia (Beutter *et al.*, 2001).

1.2.9 Red cell distribution width RDW:

The red cell distribution width (RDW) is a mathematical description of the variation in RBC size; a high RDW indicates greater variation in RBCs size normal range of RDW = (CV) 12.8-1.2% (SD) 42.5-3.5fl (Kern,2002).

1.2.10 Hemoglobin (Hb):

It is the iron- containing oxygen-transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates (Sidell *et al.*,2006).

1.2.10.1 Structure of hemoglobin:

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular protein (Steinberg,2001).

Hemoglobin molecule is an assembly of four globular protein subunits.Each subunits is composed of a protein chain tightly associated with a non – protein prosthetic hemegroup. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin foldarrangement (Vanbeekvelt *et al.*,2001).

1.2.10.2Types of hemoglobin:

Hemoglobin variants are a part of the normal embryonic and fetal development.

-In the embryo: Gower 1,Gower 2($\alpha_2 \epsilon_2$),Hemoglobin Portland I ($\zeta_2\gamma_2$) and Hemoglobin Portland II($\zeta_2 \beta_2$)

-Fetus develops Haemoglobin –F(Hb-F) which contain Two alpha and Twogamma ($\alpha_2\gamma_2$) globin chains.(Chen and Keda,2008).

- After birth:

Hemoglobin A ($\alpha_2\beta_2$) the most common with a normal amount over 95%.

Hemoglobin A₂ ($\alpha_2 \delta_2$) – δ chain synthesis begins late in the third trimester and in adult it has a normal range of 1.5-3.5%.

Hemoglobin F ($\alpha_2 \gamma_2$) in adult hemoglobin F is restricted to a limited population of red cells called F-cells (Storz *et al.*,2013).

A low hemoglobin level is referred to as anemia or low red blood count. Higher than normal hemoglobin level can be seen in people living at altitudes and in people who smoke (Ernest and Jill,2006).

1.2.11 Leukopoiesis:

Leukopoiesis is the formation and development of TWBCs occurs in same location as erythrocyte, with exculpation of lymphocyte (Hoffbrand *et al.*,2001).

1.2.12 Granulopoiesis:

Defined as process by which mature granulocyte produced in the bone marrow in myeloid series. It occurs primarily within bone marrow (Basu *et al.*,2004).

1.2.12.1 Stage of Granulopoiesis:

-The myeloblast size 14-18Mm that has large nucleus. Basophilic cytoplasm.Nucleoli are typically prominent while two or three is the usual number (Mitchell *et al.*,2007)

-promyelocyte cytoplasmic granules and a slightly more coarse appearance of the chromatin (Hoffbrand and Moss,2011).

-myelocyte ,prominent cytoplasmic granules, Nucleoli are no longer present.

-metamyelocyte (Neutrophilic Metamyelocyte):Size about 15Mm, nucleus become indented and take the kidney.

-Polymorph nuclear neutrophilic(segmented neutrophilic):Diameter 8-10Mm,nuclear materias divided into 3-5 lobes and cytoplasm pink colour.

-polymorphonuclear eosinophils: have diameter of up to 12-17 μ m.two nuclear lobes. The cytoplasm has a pale hue and contains many granules (Young *et al.*,2006)

-The polymorphnuclear basophils : granules are intensely basophilic, and tend to overlies and obscure the nucleus under the microscope when stained (Young *et al.*,2006).

1.2.12.2 White blood cell count:

Used to help in diagnosis of leukemia,bacterial and viral infection. Dilution whole blood 1:20 in 2% glacial acetic acid.Then count the TWBCs in four center squares and calculate according to formula:

$$\text{TWBCs count} = \frac{\text{sum} \times \text{dilution factor}}{\text{Area} \times \text{depth}}$$

1.2.12.3 Monocyte-macrophage series:

Monoblast : large cell, cytoplasm small in the amount, nucleus large in size and chromatin open (Dacies and lewis,2011).

Promonocyte: It is similar in size to the promelocyte. Irregularly shape cytoplasm, nucleus indented and nucleoli not seen.

Mature monocyte: irregularly shaped nucleus with a relatively fine chromatin pattern. Cytoplasm is abundant and of pale grey-blue tint.

Macrophage: 15-18µm in diameter.They have one or more oval nuclei, and irregular or oval cytoplasm outline (Dacies and Lewis,2011).

1.2.12.4Lymphocyte development (Lymphopoiesis):

The lymphopoiesis the generations of lymphocyte occurred in foci in the bone marrow and in the thymus are engaged in particularly rapid proliferation which is not specifically related to antigenic stimulation (Birbrair and Frenette,2016)

1.2.12.4.1lymphoid series:

-Lymphoblast: It small size10-20, round to oval nucleus,more condensed chromatin, cytoplasm is scanty and nucleoli seen (Kumar *et al.*,2013).

-Prolymphocyte: Size 10-18, slightly more clumped chromatin, lessening of nucleolar prominence (Gillian,2011).

-Large lymphocyte is between 10-20 μm in diameter, a nucleus is deeply colored and is composed of dense aggregates of chromatin (Farlex,2012).

-Small lymphocyte are between 7-10 μm in diameter with around or slightly indented heterochromatic nucleus (Miller,2003).

1.2.13Thrombopoiesis:

Platelet are produce in the bone marrow by fragmentation of the cytoplasm of megakaryocyte, one of the largest cells in the body (Hoffbrand and Moss,2011).

The promegakaryocyte is the next stage in the sequence of end maturation, and is large than it is precursor because has undergone end replication.

Promegakaryocyte: 30-90 μm in diameter and contain 4-16 nuclear lobes with coarsely clumped chromatin.

Platelets have no nucleus, they are fragment of cytoplasm that are derived from the megakaryocyte of the bone marrow, and then enter the circulation. Structures 2-3 μm in greatest diameter, cytoplasm blue color and contain small red-purple granules (Machlus *et al.*, 2014).

1.2.14 Platelets count:

Platelets are the smallest type of the blood cells, they are important in blood clotting when bleeding occur the platelets swell, clump together and form a sticky plug that help to stop the bleeding (Alberts and Bruce,2005).

Platelets count = sum \times dilution factor

Area \times depth

1.2.14.1 Mean platelet volume:

It is measurement of the average size of platelets. Abnormal low MPV values correlate with thrombocytopenia when it is due to impaired production as in Aplastic anemia (Liu *et al.*,2012)

1.2.15 Pregnancy:

Pregnancy is the carrying of one or more offspring known as a fetus or embryo inside the womb of female. Childbirth usually occurs about 38 weeks after conception, in women who have a menstrual cycle length of four weeks, this approximately 40 weeks from the last normal menstrual period (LNMP) (Widmaier *et al.*, 2006).

1.2.15.1 Pregnancy trimester:

1.2.15.1.1 First trimester:

The events that lead to pregnancy begin with conception, in which the sperm penetrates the egg produced by an ovary. The zygote (fertilized egg) then travels through the woman's fallopian tube to the uterus, where it implants itself in the uterine wall. The zygote is made up of a cluster of cells formed from the egg and sperm. These cells form the fetus and placenta (pregnancy condition information, 2016).

1.2.15.1.2 Second trimester:

During the second trimester the baby is growing quickly between 18 and 22 weeks of pregnancy, mother can see the baby progressing uses ultrasound. Vernix and lanugo keep the fetus skin from chapping in the amniotic fluid. Most of the brain neurons present by 24 weeks and the fetus reacts to sound: the fetus becomes 30 cm in length and 820 gm in weight (Berk, 2011).

1.2.15.1.3 Third Trimester:

The 26 to 40 weeks of third trimester is the period in which fetal organs complete their prenatal maturation, during it, the growth rate decelerates 3200g and is about 50 cm long term is the interval from 37 to 42 weeks normal labor, rhythmic uterine contraction, and birth occurring during this period (Carel *et al.*, 2008).

1.2.15 .2 Physiological change associate with pregnancy:

Plasma volume increase progressively throughout normal pregnancy. Platelet count tends to fall progressively during pregnancy, although it usually remains within normal limits (Rodger *et al.*,2015)

Changes in the cardiovascular system in pregnancy are profound and begin early in pregnancy, such that by eight weeks gestation, the cardiac output has already increased by 20%.The primary event is probably peripheral vasodilatation .This is mediated by endothelium- dependent factors, including nitric oxide synthesis, up regulated by estradiol and possibly vasodilator prostaglandins(PGI₂) (Ramsay,2010).

Peripheral vasodilatation leads to a 25-30%fall in systemic vascular resistance, and to compensate for this, cardiac output increase by around 40% during pregnancy.Blood pressure levels in the first and second trimesters but increase to non –pregnant level in the third trimesters (Rodger *et al.*,2015).

The increased renal blood flow leads to an increase in renal size of 1-5.5cm, reaching the maximal size by mid-pregnancy. The kidney pelvis and calycesystems dilate due to mechanical compressive forces on the ureters (Cheung and Lafagette,2013).

1.2.15.3 Hematological change associate with pregnancy:

During pregnancy the plasma volume increase by 50% and red blood cell volume increase only by 20-30 %.(Guyton and Hall,2005).

Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to higher proportion of young RBCs which are larger in size), MCV does not change significantly during pregnancy and heamoglobin concentration < 9.5 g/dL in association with a mean corpuscular volume < 84 fl probably indicate co-existent iron deficiency or some other pathology (Crocker *et al.*,2000).

White blood cell count is increased in pregnancy with the lower limit of the reference range being typically 6,000/cumm. Leucocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state (Jessica *et al.*,2007).

Lymphocyte count decrease during pregnancy through the first and second trimester and increase during the third trimester .there is an absolute monocytosis during pregnancy, especially in the first trimester, but decrease as gestation advances (Kline *et al.*,2005).

Eosinophil and basophil counts, however don't change significantly during pregnancy (Edlestam *et al.*, 2001).

Platelets count does decrease during pregnancy, particularly in the third trimester it is partly due to hem dilution and partly due to increased platelet activation and accelerated clearance while platelets volume distribution width increase significantly and continuously as gestation advances, for reasons cited before (Shehlata *et al.*, 2000).

1.2.15.4 Haemostatic change associate with pregnancy:

Significant physiological changes during pregnancy result in a hypercoagulable and hypofibrinolytic state that serves to protect the mother from bleeding complications at the time of placental separation (Miller and Laffan,2015).

In normal pregnancy, there is a marked increase in the procoagulant activity in maternal blood characterized by elevation of factor VII,X,VIII,fibrinogen and von will brand factor, which is maximal amount term.This associated with an increase in prothrombin fragments (PF1+2) and thrombin-anti thrombin complexes.The overall fibrinolytic activity is impaired during pregnancy, but returns rapidly to normal following delivery this largely due to placental derived plasminogen activator inhibitor type 2(PAI-2), which is present in substantial quantities during pregnancy (Benjamin,2004).

1.2.15.5 Infection associated with pregnancy:

Pregnancy can make more susceptible to certain infections. Pregnancy may also make these infections more severe. Even mild infections can lead to serious illness in pregnant women. Some infection that occur during pregnancy can lead to miscarriage, pre term labor, or birth defect. Viral and bacterial infections can develop in any one, but certain infections are more likely to occur in pregnant women (Nicole,2016).

Bacterial infection can affect pregnant women from implantation of fertilized ovum through the time of delivery and per partum period.

Urinary tract infections is common in pregnancy. Asymptomatic bacteriuria develops in 10-15% of pregnant women and can lead to complication such as pyelonephritis and premature labor (Darvin,2016).

1.2.16 Anemia:

Anemia is a decrease in the total amount of red blood cells or hemoglobin in the blood or allowed ability of the blood to carry oxygen (Rodak and Bernatted,2007).

The symptoms can be related to an underlying cause or the anemia itself. Most people feeling of weakness or tired and sometimes poor concentration (Saimak and Nabili,2009).

The most useful classification is that based on red cell indices and divides the anemia into microcytic, normocytic and macrocytic anemia (Hoffbrand *et al.*, 2005).

1.2.17 Common type of anemia during pregnancy:

There are several types of anemia's that may occur in pregnancy:

1.2.17.1 Iron deficiency anemia:

An iron deficiency anemia account for 75-95 % of cases of anemia in pregnant women. A women who is pregnant often has insufficient iron stores to meet the demands of pregnancy. Pregnant women are encouraged to supplement their diet with 60 mg of elemental iron daily.

An MCV less than 80 mg/dL and hypochromia of RBCs should prompt further studies, including total iron-binding capacity, ferritin levels and Hb electrophoresis if iron deficiency is excluded (Ebrahim *et al.*,2010).

Approximately 15% to 25% of all pregnancies experience iron deficiency. During pregnancy, need double the amount of iron that non-pregnant women need. Body needs this iron to make more blood to supply oxygen to baby. If don't have enough iron stores or get enough iron during pregnancy that lead to iron deficiency anemia (Ebrahim *et al.*,2010).

1.2.17.1.1 Iron metabolism:

Total iron content, about 400 mg are the body devoted to cellular proteins that use iron for important cellular processes like storing oxygen (myoglobin) or performing energy-producing redox reactions (cytochromes) (Camaschella and Schrier,2011).

Absorption and Storage of iron occurs in two forms-ferritin and haemosiderin. Ferritin is normally predominant and haemosiderin is an iron storage complex, It found within cells and appears to be a complex of ferritin, denatured ferritin and other material (Fischbach *et al.*,2016).

Iron is mainly absorbed in the duodenum and upper jejunum. A transporter protein called divalent metal transporter 1 (DMT1) facilitates transfer of iron across the intestinal epithelial cells (Fuqua *et al.*,2012).

1.2.17.1.2 Causes of iron deficiency anemia:

It can be caused by increased iron demand, loss or decreased iron intake, and can occur in both children and adult. The cause of chronic blood loss should be considered, according to the patient sex, age and history. In women of childbearing age, heavy or long menstrual period can also cause mild iron-deficiency anemia (Rangarajan *et al.*,2007).

Pregnancy: low iron levels are a common problem for pregnant women. The growing fetus needs a lot of iron, which can lead to an iron deficiency.

Also, a pregnant woman has an increased amount of blood in her body. This larger volume of blood demands more iron to meet its needs (Bermejo and Garcia,2009).

1.2.17.1.3 Diagnosis of iron deficiency anemia:

Diagnosed by routine blood tests, include a complete blood count (CBC). Sufficiently low hemoglobin (Hb) by definition makes the diagnosis of anemia, and a low hematocrit value is also characteristic of anemia if the anemia is due to iron deficiency, low MCV, low mean corpuscular hemoglobin and or mean corpuscular hemoglobin concentration (Stephen *et al.*,2009).

Low serum iron and ferritin levels with an elevated TIBC are diagnostic of iron deficiency. While a low serum ferritin is virtually diagnostic of iron deficiency (Hempel and Bollard 2016).

1.2.17.2 Megaloblastic anemia:

Is an anemia that results from inhibition of DNA synthesis during red blood cell production .when DNA synthesis is impaired, the cell cycle cannot progress from the G₂ growth stage to the mitosis (M) stage. The defect in red cell DNA synthesis is most often due to hypo vitaminosis, specifically a deficiency of vitamin B₁₂ and or folic acid (Berkowitz,2012).

It is characterized by the appearance in the bone marrow of morphologically abnormal nucleated red cell precursors, which Ehrlich in 1880 called megaloblast(Hoffbrand *et al.*,2001).

1.2.17.2.2 Diagnosis of megaloblastic anemia:

Features suggestive of a megaloblastic anemia include hyper-segmented neutrophil nuclei (more than five segments), oval macrocytes and mild leucopenia and thrombocytopenia in severe cases. Red cell folate assay 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. B₁₂ levels fall in pregnancy, but this is not thought to represent a true tissue deficiency (Pavord and Hunt,2010)

1.2.17.3 Vitamin B₁₂ deficiency Anemia:

Vitamin B₁₂ deficiency anemia, of which pernicious anemia is a type, is a disease in which not enough red blood cells are present due to a lack of vitamin B₁₂. Although pernicious anemia technically refers to cases resulting from not enough intrinsic factor, it is often used to describe all cases of anemia due to not enough vitamin B₁₂ (Hvas and Nexø,2006).

Women who are vegans (who eat no animal products) are most likely to develop vitamin B₁₂ deficiency. Including animal foods in the diet such as milk, meat, eggs, and poultry can prevent vitamin B₁₂ deficiency. Strict vegans usually need supplemental vitamin B₁₂ by injection during pregnancy.

Lack of vitamin B₁₂ in the body leads to poor formation of blood cells; in condition like pregnancy where the entire body goes through a dramatic change, good amount of this crucial vitamin are required. Vitamin B₁₂ deficiency anemia is to induce preterm labor. Sometimes, pure red cell aplasia occurs during pregnancy without any apparent explanation and typically disappears following delivery (Douglas and Hirschmann,2007).

1.2.17.4 Folate-deficiency anemia:-

Folate, also called folic acid, is a type of B vitamin. The body needs folate to produce new cells, including healthy red blood cells. During pregnancy, women need extra folate. But sometimes they don't get enough from their diet. When that happens^s, the body cannot make enough normal red blood cells to transport oxygen to tissues throughout the body. Folate deficiency during human pregnancy has been associated with an increased risk of infant neural tube defect. Such deficiency during the first four weeks of gestation can result in structural and developmental problems. NIH guidelines to recommend oral B vitamins supplements to

decrease these risks near the time of conception and during the first month of pregnancy (Czeizel *et al.*, 2013).

Folate requirements are increased by 200-300µg to about 400µg daily in normal pregnancy partly because of transfer of the vitamin to the fetus, but mainly because of increased folate catabolism due to cleavage of folate co-enzyme in rapidly proliferating tissue at the C₉-N₁₀ bond. A number of consequences of folate deficiency in pregnancy have been described including antenatal and post partum hemorrhages, prematurity and congenital malabsorption in the fetus. These have not been fully established. But recent studies have shown that prophylactic folic acid therapy reduces the incidence of neural tube defect (Hoffbrand *et al.*, 2001).

Folate, forms of which are known as folic acid and vitamin B₉, is one of the B vitamins. The recommended daily intake level of folate is 400 micrograms from foods or dietary supplement. It is also used as a supplement by women during pregnancy to prevent neural tube defect (NTDs) in the baby (Fenech, 2012).

Sources of folate: widely distributed in plant and animal tissue. The richest sources are liver, kidney, yeast and fresh green vegetables. Folate is absorbed from the duodenum and upper jejunum, and to a lesser extent from the lower jejunum and ileum (Bibbins *et al.*, 2017).

1.2.18 Complete blood count CBC:

Full blood count is a very common clinical procedure and often the (starting point) for most medical investigations (Smellie *et al.*, 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 diagnosis.

impression to be formed directing further, more specific testing (Greer *et al.*, 2003).

Most blood counts today include a CBC count and leukocyte differential count (LDC) (Buttarell and Plebani 2008).

1.3 Previous studies:

Many researches were conducted in the world wide to evaluate hematological profile in normal pregnancy. Hematological profile of health pregnant women in Ibadan, South-Western Nigeria: The following hematological indices: TWBCs, platelets count, RBCs, HCT and PDW of women between the trimesters showed statistical significant (p value < 0.001 in each case). The TWBCs is inversely proportional to the PCV and the MCV in the pregnant women was slightly raised. In this study, pregnancy is characterized by high values of hemoglobin parameters in trimester three and there are statistical significant in other parameters in pregnant women compared with non pregnant women (Akingbola *et al.*,2009).

Other study conducted in Sudan a study revealed that there were significant decreased in RBCs count, hemoglobin (Hb) ,MCV,MCH,MCHC and PCV of Sudanese pregnant women compared to non-pregnant women (p value < 0.05).TWBCs count was increased significant(p value < 0.05) in contrast platelets count significantly lower than the normal control(p value < 0.05) (Algari,2013).

Other study about hemoglobin level ,RBCs indices and iron status in pregnant females in Sudan, the results showed that 8(10%) out of 80 pregnant females of them had low Hb level, while 72(90%) had normal Hb level. RBCs indices showed 62(77.5%) mothers had normal MCV, while 18(22.5%) mothers had low.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 (Abdelgader *et al.*,2014).

Tiwar (2012) found that iron deficiency anemia (IDA) is the most common cause of anemia in pregnancy in Indians. Studies from developed countries recommend iron supplementation based on serum ferritin levels. Hematological indices in pregnancy applications: an evaluation in healthy pregnant Jamaican women. The results indicate that haemoglobin concentration, packed cell volume and red blood cell count were highest in the first trimester, reaches its lowest point in the second trimester and begins to rise again in the third trimester. In contrast, the mean corpuscular volume and the mean cell hemoglobin had the lowest value in the first trimester, rose to its highest value in the second trimester and then started to decline in the third trimester. The mean corpuscular hemoglobin concentration however, remained fairly constant throughout pregnancy. The white blood cell count changed in a similar way as the mean corpuscular volume and the mean cell hemoglobin. The platelet count decrease from the first trimester to the third trimester (James *et al.*, 2008).

Other study done in Sudan determination of complete blood cell count in Sudanese pregnant women in the second trimester in Khartoum locality result showed that: Hb significantly decreased. MCV and neutrophil significantly increased and no significant difference in CBC according to history of abortion (Abdalla, 2015).

1.4 Rationale:

Pregnancy is often associated with physiological changes and haemostatic change which may lead to pregnancy complication so, blood cells count is important of pregnancy to detect complication.

Pregnancy in developing countries have high mortality ratio and higher risk specially young adolescent who face risk of complications of death as a result of pregnancy than older women (WHO,2016).

Anemia during pregnancy is a large health problem in Sudan, where pregnant women in different regions of Sudan are more susceptible to anemia; it may be due to physiological change or due to increase of nutritional demands (Abdelrahman *et al*,2012).

1.5 Objectives:

1.5.1 General objective:

To evaluate complete blood cell count of Sudanese pregnant women at the third trimester in Hassahissa and Aboaser hospital .

1.5.2 Specific objectives:

-To compare between Hb, HCT, RBCs, MCV, MCH, MCHC, TWBCs, neutrophil, lymphocyte percentage, platelets, MPV and PDW in pregnant and non-pregnant women.

-To compare between regular visits to clinic, abortion, and supplemental medication used by pregnant women in third trimester.

Chapter Two

Materials and Methods

2.1 Study design area and duration:

This was case control study conducted in obstetric Hassahissa hospital and Aboasher hospital from February to March 2016 to determine the CBC of pregnant women at the third trimester.

2.2 Sample size:

The study included 250 samples. Include 150 pregnant women at third trimester and 100 non pregnant women in different age group.

2.3 Study population:

Sudanese pregnant women in third trimester attended to Hassahissa obstetric hospital and Aboasher hospital in Gazera state and control group

2.3.1 Inclusion criteria:

Pregnant women with apparently normal pregnancy and without disease that may cause hematological change .

2.3.2 Exclusion criteria:

Pregnant women at first or second trimesters . Presence of any diagnostic diseases such as: typhoid fever, malaria, anemia ,malignancy and pervious blood transfusion were excluded.

2.3.3 Sampling technique:

Non probability informed consent sampling.

2.4 Method of data collection:

Data was collected using questionnaire which was specifically designed to obtain information about demographic and clinical data that helped in either including or excluding certain individual in or from the study respectively.

2.5 CBC determination:

Full automated cell counter (Mindary BC-3000 plus):

2.6 Blood test and procedure:

2.6.1 Sampling collection:

Using a sterile disposable syringe 2.5 ml of blood were collected and drained in EDTA container, mixed gently. The container labeled clearly with the participants number.

2.7 Method of diagnosis:

2.7.1 CBC measurement:

Principle of hematologic analyzer impedance (resistance to current flow) . In this method small sample of the blood is aspirated into a chamber and diluted with an isotonic saline solution. The analyzer prepares two dilutions one lysed and the other unlysed, after the first dilution is measured, the white blood cell and hemoglobin value are displayed on the screen of the instrument, the analyzer processes the second dilution, which measures RBCs, MCV and platelet count. Small portion of the diluted in each bath is allowed to flow past a small aperture, an electrical current is produced in each aperture by two electrodes (Chapman and Abraham 2000).

2.7.1.1 General principle of Mindary:

The two independent measurement methods used in this analyzer are:

The coulter method determining the TWBCs RBCs and platelets count.

.The colorimetric method for determining the Hb during each analysis cycle, the sample is aspirated diluted and mixed before the determination for each parameter is performed.

2.7.1.2 Measurement of WBCs,RBCs,PLT:

Automated counting method for RBCs has been based originally on electrical impedance or later on light-scattering techniques. The hemoglobin concentration is measure optically using the solution in the WBCs bath .The lysing agent contains potassium cyanid that react with hemoglobin to form cyanamethemoglobin The color intensity, measure

in separate cavetti's read spectrophotometrically at 540 nm.HCT can calculate automatically by multiplying the RBCs count. by MCV. The MCV is measured by electric cell count.MCH is calculated by dividing hemoglobin concentration by the RBCs count. MCHC is calculating by hemoglobin by HCT. Platelet count is measured by impedance counting (Zandeki *et al.*,2007)

2.8 Quality control (QC):

Quality control (QC) consists of strategies and procedures that measure the precision and stability of the analyzer.

2.9 Ethical consideration:

The study was approved by medical ethical committee of Medical Laboratories-Science -Sudan University of Science and Technology and Hassahissa obstetric hospital –Aboasher hospital .Verbal informed consent was obtained from the participant after they have been informed with the objectives, benefits and expected outcome of study.

2.10 Statistical analysis:

Statistical package of social sciences SPSS version 16 software programs was used for statistical analysis Independent T-test and one way ANOVA were used to obtain *P-value* significant level was set at ≤ 0.05 .

Chapter three

Results

This study was carried out in Hassahissa obstetric hospital and Aboasher hospital at Gazera state during the period from February to march 2016 to measure CBC of pregnant women at third trimester.

(21-30) years were showed that most frequent group in (98 / 150,65.3%) in pregnant women and was(57/100, 57%) in non-pregnant women while the least frequent group in study volunteers was (≤ 20) 17 (11.4%) and (>30) years 35 (23.5%) in pregnant women and was (≤ 20) 9 (9%) and (> 30) years 34 (34%) in non-pregnant women.

Table (3.1) Distribution of age group among study population:

Age group year	Pregnant in 3 rd trimester frequency	Non pregnant frequency
≤ 20	17 / 150,11.4%	9 / 100,9%
21-30	98 / 150,65.3%	57 / 100,57%
> 30	35 / 150,23.5%	34 / 100,34%
Total	150	100

The result showed that there was a significant decrease in Hb,HCT,RBCs MCHC ,lymphocyte ,mix and Platelet of pregnant women compared to control, a significant increase in TWBs ,neutrophil, PDW in pregnant women compared with control. No statistical correlation of MCV, MCH and MPV of pregnant women compared to control.

Table (3.2) Comparison of complete blood count between pregnant and non pregnant women:

Parameter	Mean \pmSD of pregnant women	Mean \pm SD of non pregnant women	<i>P .value</i>
Hb g/dl	10.5 \pm 1.2	10.9 \pm 1.2	0.010
HCT%	36.5 \pm 3.8	37.9 \pm 3.5	0.000
RBCs $\times 10^{12}$ / l	4.2 \pm .45	4.4 \pm 4.3	0.004
MCV fl	86.7 \pm 6.4	85.5 \pm 5.2	0.140
MCH Pg	24.3 \pm 2.2	24.2 \pm 2.5	0.85
MCHC %	27.8 \pm .89	28.2 \pm 1.5	0.007
TWBCs $\times 10^9$ /l	8.1 \pm 2.7	7.1 \pm 3.1	0.005
lymph %	25 \pm 9.4	31.1 \pm 10.1	0.000
neutrophil %	66.1 \pm 9.8	59.1 \pm 11.1	0.000
Mix %	7.8 \pm 2.2	7.5 \pm 2.2	0.012
platelet $\times 10^9$ /l	225.8 \pm 67.1	272.1 \pm 97.2	0.000
MPV fl	8.5 \pm 1.1	9.1 \pm 6.8	0.302
PDW fl	15.6 \pm .35	15.2 \pm 1.1	0.000

There was no statistical different between age group of pregnant women and Hb, HCT, RBCs, MCV, MCH, MCHC, TWBCs, neutrophil, lymphocyte mix, Platelet, MPV and PDW p-value of Hb of age group ≤ 20 and 21-30 (*P.Value* =0.45), HCT of age group ≤ 20 & > 30 (*P.Value* = 0.24).

Table (3.3) Effect of age group on TWBCs, RBCs, Hb, HCT and PLTs during pregnancy:

Parameter	≤ 20 Mean \pm S.D	21-30 Mean \pm S.D	> 30 Mean \pm S.D	<i>P .value</i>
Hb g/dL	10.3 \pm 1.4	10.5 \pm 1.3	10.7 \pm 1.1	0.45
HCT%	35.5 \pm 3.6	36.4 \pm 4.5	37.3 \pm 3.9	0.24
RBCs $\times 10^{12}$ /L	4.3 \pm 0.52	4.1 \pm 0.43	4.2 \pm 0.47	0.18
MCV fl	82.9 \pm 7	87.3 \pm 5.9	87.2 \pm 7.1	0.03
MCH pg	23.1 \pm 2.6	24.9 \pm 2.1	24.5 \pm 2.3	0.089
MCHC%	27.2 \pm 0.9	27.8 \pm 0.8	28 \pm 0.8	0.023
TWBCs $\times 10^9$ /L	7.9 \pm 2.5	8.3 \pm 2.9	7.7 \pm 2.1	0.44
neutrophil%	62 \pm 13.5	67.1 \pm 9.3	65.1 \pm 8.9	0.11
lymphocyte%	28.3 \pm 12.7	24.2 \pm 9.1	25.6 \pm 8.2	0.23
mix%	8.5 \pm 2	7.7 \pm 2.3	7.6 \pm 2.2	0.31
platelet $\times 10^9$ /L	232 \pm 39.2	228 \pm 74.6	216 \pm 54.3	.062
MPV fl	8.7 \pm .87	8.5 \pm .88	8.3 \pm 1.4	0.50
PDW fl	15.7 \pm 0.3	15.5 \pm 0.3	15.6 \pm 0.32	0.29

Hb, HCT, RBCs, MCV, MCH and MCHC showed increased levels of pregnant taken supplement when compared with those not taken supplement ($p \text{ value} \leq 0.05$) and no statistical correlation in mean of TWBs, lymphocyte neutrophil mix, platelet MPV and PDW of pregnant take supplementation when compared with those not take supplementation ($p \text{ value} > 0.05$).

Table (3.4) Relation between CBC and supplement taken among pregnant women:

Test	Supplement taken Mean± SD	Supplement untaken Mean± SD	<i>P. value</i>
Hb g/dl	10.7 ± 1.2	9.6 ± 1.1	0.000
HCT%	37.1 ± 3.7	33.7 ± 3.7	0.000
RBCs×10 ¹² /l	4.2 ± .45	4.1 ± .38	0.032
MCV fl	87.2 ± 6.5	84.2 ± 5.2	0.047
MCH Pg	24.4 ± 2.2	23.2 ± 1.9	0.016
MCHC %	27.8 ± .86	27.4 ± 1.01	0.034
TWBCs×10 ⁹ /l	8.2 ± 2.5	8.1 ± 3.7	0.91
lymph %	24.6 ±9.0	27.2 ±11.4	0.23
neutrophil %	66.2 ± 10.1	65.9 ± 8.6	0.88
mix %	7.9± 2.2	7.2± 2.2	0.18
platelet×10 ⁹ /l	229 ± 50.9	229 ± 50.9	0.81
MPV fl	8.5 ± .79	8.5 ± .79	0.97
PDW fl	15.6 ± .36	15.6 ± .36	0.22

There was no different between history of abortion and Hb, HCT,RBCs ,MCV, MCH, MCHC and platelet count for example. TWBCs (*P.Value* = .98), RBCs (*P.Value* = .59).

Table (3.5) Effect of history of abortion on cell blood count during pregnancy:

Parameter	With history of Abortion Mean ± S.D	Without history of abortion Mean ± SD	<i>P. value</i>
Hb g/dl	10.5 ± 1.2	10.5± 1.2	0.95
HCT%	36.5 ± 4.2	36.5 ± 3.7	0.98
RBCs × 10 ¹² /l	4.1 ±.43	4.2 ± .46	0.59
MCV fl	88 ±5.9	86 ± 6.5	0.18
MCH Pg	24.5 ± 2.2	24.2± 2.2	0.39
MCHC %	27.7 ± 1.0	27.8 ± .85	0.76
TWBCs ×10 ⁹ /l	8.1 ± 3.1	8.1 ± 2.5	0.98
lymph %	26.1± 7.9	24.6 ± 9.8	0.41
neutrophil %	64.7 ± 8.3	66.6 ± 10.3	0.29
mix %	7.9 ± 2.1	7.7 ± 2.3	0.63
platelet × 10 ⁹ /l	209.6±52.9	231.3±70.7	0.08
MPV fl	8.6 ± .72	8.4 ±1.1	0.28
PDW fl	15.6 ± .33	15.4 ± .35	0.20

There was no different between CBC and regular follow up.

Table (3.6) Relation between CBC and visit to clinic:

Parameter	Visit to clinic Mean \pm S.D	No visit to clinic Mean \pm SD	<i>P. value</i>
Hb g/dl	10.6 \pm 1.3	10.2 \pm .89	0.06
HCT%	36.6 \pm 4.0	36.1 \pm 3.1	0.47
RBCs $\times 10^{12}/1$	4.2 \pm .44	4.1 \pm .48	0.70
MCV fl	87 \pm 6.6	85.9 \pm 5.6	0.39
MCH Pg	24.4 \pm 2.3	23.9 \pm 2.0	0.27
MCHC %	27.8 \pm .91	27.6 \pm .80	0.18
TWBCs $\times 10^9/1$	8.3 \pm 2.8	7.7 \pm 1.9	0.35
lymph %	25.0 \pm 10.0	24.9 \pm 6.2	0.91
neutrophil %	66.2 \pm 10.3	65.8 \pm 7.5	0.82
mix %	7.7 \pm 2.2	7.9 \pm 2.2	0.69
platelet $\times 10^9/1$	224.4 \pm 63.8	231.4 \pm 80	0.61
MPV fl	8.5 \pm 1.1	8.4 \pm .76	0.72
PDW fl	15.5 \pm .35	15.6 \pm .34	0.63

Chapter four

Discussion, Conclusion, and Recommendations

4.1 Discussion:

This study was carried out in Hassahissa obstetric hospital and Aboasher hospital at Gazera state during the period from February to March 2016 to measure CBC of pregnant women at third trimester. This study aimed to determine the hematological parameter of the pregnant female to monitor illness pregnant women. The present study showed that age group (20-30) years were the most frequent while ≤ 20 years showed least frequent in both study population.

The results showed that the mean of Hb, HCT, RBCs, MCHC was significant decrease in pregnant women compared to control. These result agreed with the results of a study in Sudan obtain by (Mohamed and Ibrahim, 2016). Also this result agreed with the results of study in China pregnant women the result characterized by lowest values of hematocrit, Hemoglobin concentration compared with control (Shen *et al.*, 2010). This result disagreed with (Khlil, 2012) observed that Hb concentration of pregnant women did not vary significant from that of non-pregnant women. Decline in Hb may be due to an increased demanded for iron and nutrient as pregnancy progresses (Somendra *et al.*, 2016).

The platelets count, MPV and lymphocytes decreased significantly 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. Also This result was agree with previous study conduct by (Akinbami *et al.*, 2013) who found significant decrease of platelet in pregnant women when compared with 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. MCV, MCH showed no statistical correlation while TWBCs, neutrophil and PDW showed significant increase when compared between non pregnant women. The result agree with (Pughikumo *et al.*,2015) who reported that TWBCs count increase in pregnant women compared with non-pregnant women. In present study there was no significant different between age groups in Hb, HCT,RBCs, TWBCs while platelet insignificant decrease in age group (> 30). Similar result obtained by (Elsidig,2017) who found age group (20-35) years most frequent

This study showed that there was significant increase in mean of Hb, HCT, RBCs, MCV,MCH, MCHC of pregnant women take supplementation when compared with those who did not take supplementation, while there were no significant correlation in TWBCs, lymphocyte, neutrophil, platelet, mix and MPV of pregnant take supplementation when compared with those who did not take supplementation. This result agree with result obtains by Kumar in Iranian pregnant women in Urmia. The low prevalence of anemia in her study may be related to more frequent iron supplementation consumption (Kumar *et al.*,2013).

In present study there was no statistical correlation in Hb, HCT, RBCs, MCH, MCHC, TWBCs, mix, MPV in the pregnant women with history of abortion or not. This result agreed with study conducted by (Nasor,2015) who report that no significant effect of abortion and disagree with result obtained by (Ekram,2017) who found the effect of some parameters of CBC. MCV and lymphocyte percentage was insignificant increased while platelets and neutrophil percentage was insignificant decreased in pregnant women with history of abortion and pregnant women with not abortion.

In present study there was no statistical correlation in Hb ,HCT,RBCs, MCV,MCH,MCHC,TWBCs, neutrophil, lymphocyte, mix, platelet, MPV and PDW of pregnant who's regularly follow up when compared with those irregular follow up. Similar result obtain by (Abdalla, 2015) who found no significant on CBC according to regular clinic visit.

4.2 Conclusions:

1- Decrease in Hb, HCT, RBCs, MCHC, lymphocyte percentage ,mix and platelet in pregnant women compared to non-pregnant while TWBCs, neutrophil percentage and PDW was increase in pregnant women compared with control.

2- Pregnant women who taken supplementation the Hb, RBCs, MCV, MCH,MCHC TWBCs and neutrophil percentage was increased while platelet and lymphocyte was decreased in pregnant women taken supplementation with that no taken supplementation.

3- No different in Hb, HCT, RBCs, MCH, MCHC, TWBCs, mix and MPV in pregnant women with history of abortion or not and regular follow up. While MCV and lymphocyt percentage was increased However platelet and neutrophil percentage was decreased in pregnant women with history of abortion or not .

4.3. Recommendation:

- Regular measurement of CBC during pregnancy.
- Taken supplement of pregnant women especially in third trimester.
- Nutritional sessions should be provided for the community through multimedia, schools, health visitors and other health providers.
- Anemic pregnant women need further investigation in order to identify the etiology whenever possible, despite commencing the usual treatment with iron and folate.
- Another study should be conducted with a large sample size with additional other hematological parameter and further studies in differentiation of nutritional anemia's among pregnant females.

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Appendix(1)

Sudan University of Science and Technology

Collage of Graduate Studies

Assessment of blood cell count Sudanese pregnant women at Third trimester attended to Hassahissa and Aboasher hospital in Gazera state

No ()

Personal data

name.....

Age.....

Supplement (which): Yes () No ()

Histry of abortion: Yes () No ()

Visit to clinic: Yes () No ()

Result:

Hb.....g/dL Hb.....%

RBCs count..... $\times 10^{12}$ L PCV.....%

MCV.....FL MCH.....Pg MCHC.....%

TWBCs..... $\times 10^9$ L Lymphocyte.....%

Neutrophil.....% Mix.....%

Platelet count..... $\times 10^9$ MPV.....FL PDW.....FL

Date.....Signature.....

Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم أمراض الدم و المناعة الدموية

براءة أخلاقية

الاسم.....

سوف يتم اخذ عينة من الدم (3 مل) من الوريد بواسطة حقنه طعن وذلك بعد مسح منطقة العينه بواسطة مطهر.

كل الأدوات المستخدمة تأخذ العينه معقمة ومتبع فيها وسائل السلامة المعملية وسوف يتم اخطارك بالنتيجه بسريه المعلومات وان لا تستخدم الا لغرض البحث.

أوافقنا المذكور أعلاهأخذ عينة لإجراء الدراسة.

الإمضاء.....

التاريخ.....

اسم الباحث: وصال موسى احمد

0913619206

وصال

Appendix(3)

Requirments of collection:

Automated heamatological analyzer mindary

Syring and container with (EDTA) Anticoagulant

Cotton

Alcohol(75%) disinfectant.