

Chapter One

Introduction and Literature Review

1-1 Introduction:

Pregnancy is a major international health care problem, a good health insurance programs during pregnancy is now more important than ever to avoid any pregnancy complications that affect both pregnant woman and her fetus. Normal pregnancy is associated with physiological and anatomical adjustments that cause marked changes in the maternal organisms, including the composition of humeral elements of circulating blood. Many of these changes start up from the time of implantation and extend throughout pregnancy until the end of lactation. Knowledge of such changes is necessary for a proper assessment of the complications induced by pregnancy (Cunningham *et al*,1993).

In 2013, complications of pregnancy in United States resulted globally in 293,000 deaths down from 377,000 deaths in 1990. The most common causes include maternal bleeding, anemia, abortion, high blood pressure of pregnancy, maternal sepsis, urinary tract infections and obstructed labor. Approximately half of pregnant women suffer from anemia worldwide. Anemia is a major pregnancy complication and its prevalence during pregnancy differed from 18% in developed countries to 75% in South Asia. Other significant hematological changes are neutrophilia, thrombocytopenia and procoagulant factors increasing (Stephanie *et al*,2013).

1.2 Literature Review

1.2.1 Haemopoiesis

Haemopoiesis is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells. In a healthy adult person, approximately 10^{11} – 10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. The definitive haemopoiesis derives from a population of stem cells, these common precursors of endothelial and haematopoietic cells are believed to seed the liver, spleen and bone marrow and from 6 weeks until 6–7 months of fetal life the liver and spleen are the major haematopoietic organs and continue to produce blood cells until about 2 weeks after birth. The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation (Hoffbrand *et al.*, 2006).

Blood cells are divided into three lineages:

- Erythroid cells are the oxygen carrying red blood cells. Both reticulocytes and erythrocytes are functional and are released into the blood. In fact, a reticulocyte count estimates the rate of erythropoiesis.
- Lymphocytes are the cornerstone of the adaptive immune system. They are derived from common lymphoid progenitors. The lymphoid lineage is primarily composed of T and B-cells.
- Myelocytes, which include granulocytes, megakaryocytes and macrophages are derived from common myeloid progenitors, are involved in such diverse roles as innate immunity, adaptive immunity, and blood clotting (Lewis *et al.*, 2001).

As a stem cell matures it undergoes changes in gene expression that limit the cell types that it can become and moves it closer to a specific cell type. These changes can often be tracked by monitoring the presence of proteins on the surface of the cell. Each successive change moves the cell closer to the final cell type and further

limits its potential to become a different cell type. There are two points of view. For the stem cells and other undifferentiated blood cells in the bone marrow, the determination is generally explained by the determinism theory of haematopoiesis, saying that colony stimulating factors and other factors of the haematopoietic microenvironment determine the cells to follow a certain path of cell differentiation. This is the classical way of describing haematopoiesis. The other point of view is stochastic theory: Undifferentiated blood cells are determined to specific cell types by randomness. The haematopoietic microenvironment prevails upon some of the cells to survive and some, on the other hand, to perform apoptosis and die. By regulating this balance between different cell types, the bone marrow can alter the quantity of different cells to ultimately be produced. Red and white blood cell production is regulated with great precision in healthy humans, and the production of leukocytes is rapidly increased during infection. The proliferation and self-renewal of these cells depend on Growth factors. One of the key players in self-renewal and development of haematopoietic cells is stem cell factor (SCF). Absence of this factor is lethal. But there are other important glycoprotein growth factors, which regulate the proliferation and maturation, such as IL-2, IL-3, IL-6, IL-7. Other factors, termed colony-stimulating factors (CSFs), specifically stimulate the production of committed cells. Three CSFs are granulocyte-macrophage (GM-CSF), granulocyte (G-CSF) and macrophage (M-CSF). These stimulate granulocyte formation and are active on either progenitor cells or end product cells. Erythropoietin hormone is required for a myeloid progenitor cell to become an erythrocyte. On the other hand, thrombopoietin hormone makes myeloid progenitor cells differentiate to megakaryocytes (thrombocyte-forming cells). (Hoffbrand *et al*, 2006).

1.2.2 Pregnancy

Normal pregnancy is a state characterized by many physiological, hematological and anatomical changes, which may appear to be pathological in the non-pregnant state (Rao and Coslovsky, 1998).

1.2.2.1 Physiological changes associated with pregnancy

During the 279 days (40 weeks) of the pregnancy the maternal physiology changes remarkably to support the development of the fetus and to prepare the mother for labour and lactation. The uterus, for example, suffers from hypertrophy and dilatation changes, requiring increased vascularity the need for improved blood perfusion, while in the placenta due to the progressive increase, there is a related increase in the utero-placental blood flow with the evolution of pregnancy, which requires also an increase in the number of blood vessels (Cunningham *et al*, 1993).

Hormonal changes1.2.2.1.1

The physiological changes of pregnancy are controlled by an alteration in hormones secretion. The placenta produces estrogen and progesterone, the action of progesterone prevents menses and allows pregnancy to continue in early pregnancy. The elevated estrogen is increase the secretion of prolactin up to tenfold, conversely, estrogen is suppress the secretion of luteinizing hormone (LH) and folliclestimulating hormone (FSH), but other pituitary hormones such as thyroidstimulating hormone (TSH) remains nearly unchanged (Carlet *et al*, 2006).

1.2.2.1.2 Cardiovascular changes

Maternal circulation changes during pregnancy to accommodate an increase in bloodvolume of up to 50%. Due to the increase in workload, the increased blood volume peaks in the third trimester and returns to pre-pregnant state somewhere around 6–12 weeks postpartum. The systemicvascularresistance (SVR) of the blood vessels lowers due to increased levels of hormones. This decreasing SVR is an expected result of the increasing progesterone and prostaglandin levels, which relax smooth muscle, producing vasodilatation. As a result of the increased volume and

decreased resistance, cardiac output rises by 30 to 50 %, it rises quickly in the first trimester and is maintained throughout the pregnancy. Therefore, there is a normal lowering of the bloodpressure, especially in the second trimester. This sometimes causes dizziness or feeling faint in women as they rise to standing during the second trimester. Their pressure should stabilize and approach pre-pregnancy numbers by the third trimester(Blackburn, 2013).

1.2.2.1.3Renal changes

The renal system undergoes many changes to accommodate increased metabolic and circulatory requirements. The system now clears the body of both maternal and fetal waste and is affected by the increased blood volume and lowered systemic vascular resistance. Progesterone has a relaxing effect on vascular tissue, thus enhancing the renal blood flow and function. The increased plasma flow into the renal system causes the glomerular filtration rate (GFR) to rise dramatically. Renal clearance of many substances is generally elevated in pregnancy, causing lower-than-usual serum levels of the renal markers blood urea and creatinine. Increased filtration does not mean enhanced reabsorption, however. The increase in glucose load during pregnancy is often spilled into the urine and not reabsorbed. Therefore, spillage of some glucose in pregnancy is not always indicative of pathology (Blackburn, 2013).

1.2.2.2 Hematological changes

1.2.2.2.1 Blood and plasma volume

During pregnancy, there is an increase in the total blood volume of about 40 to 50% as a result of the cardiovascular system changes and the elevation in plasma volume as much as the total mass of erythrocytes and leukocytes in the circulation. Plasma volume rises gradually from the sixth week of pregnancy, increasing about 50% throughout the pregnancy reaching a peak around the 24th week. The plasma volume expansion would result in the need for increased vascular space to hold this larger volume and that would be achieved with a reduction in peripheral resistance. The increase in plasma volume is needed to meet the demand of the hypertrophied vascular system of an enlarged uterus. Plasma proteins are the most components in the blood serum, the level of some plasma proteins increase during pregnancy such as C-

reactive protein, procoagulant factors, globulins and fibrinogen. Erythrocyte sedimentation rate (ESR) rises and is affected by plasma proteins level (Rao and Coslovsky, 1998).

1.2.2.2.2 Red cell mass

The red cell mass also increases significantly, although to a lesser extent about 30% and a little later from 16th to 20th week that the plasma volume and then a hemodilution which is an adaptation of the body to transport oxygen to the fetus, since the decrease in hematocrit reduces blood viscosity and consequently the peripheral resistance. The amount of red blood cells is controlled mainly by the need for oxygen transportation, as pregnancy is a situation that demands a higher oxygen consumption increasing to need around 16%, leads consequently to an increase in the activity of erythropoietin. This increase is physiological and represents the placental hormonal control of extra-uterine environment. There is thus a moderate erythroid hyperplasia in the bone marrow and a slight increase in reticulocytes. These changes (elevation of erythropoietin level and consequently of erythrocytes) are observed after 20th week of gestation. Due to increased erythrocyte production, which exceeds the destruction, the average lifespan of red blood cells, which is approximately 120 days, is reduced in the second half of pregnancy, when production is most striking. Furthermore, the mean corpuscular volume (MCV) of erythrocytes tends to increase by decreasing its longitudinal diameter and increased thickness of its layer, making it also more spherical. The erythrocyte production and erythropoietin levels are increased during normal pregnancy, while the cell mass remains constant in relation to the body weight (Lurie and Mamet, 2000).

1.2.2.2.3 Anemia

Normally during pregnancy erythroid hyperplasia of the marrow occurs, and RBCs mass increases. However, a disproportionate increase in plasma volume results in hemodilution. Physiological anemia is the term often used to describe the fall in haemoglobin (Hb) concentration that occurs during normal pregnancy. Anemia is the commonest hematological disorder that may occur in pregnancy, the most useful classification is that based on red cell indices and divides the anaemia into microcytic, normocytic and macrocytic as well as suggesting the nature of the primary defect.

1- Microcytic Hypochromic (MCV <80fL, MCH <27 pg)

- Iron deficiency.
- Thalassaemia.
- Anaemia of chronic disease (some cases).
- Lead poisoning.
- Sideroblastic anaemia (some cases).

2- Normocytic. Normochromic (MCV 80 – 95fL, MCH ≥27pg)

- Many haemolytic anaemias
- Anaemia of chronic disease (some cases)
- After acute blood loss
- Renal disease
- Mixed deficiencies
- Bone marrow failure (e.g. post-chemotherapy. infiltration by carcinoma, etc).

3- Macrocytic (MCV > 95fL)

- Megaloblastic (vitamin B12 or folate deficiency).
- Non-megaloblastic (alcohol, liver disease, myelodysplasia, aplastic anaemia) (Hoffbrand *et al*, 2006).

1.2.2.2.3.1 Iron Metabolism changes

During pregnancy there is an increased need for iron. However, not all iron added to the maternal circulation is necessarily for the mother. There is also the need of iron for the development of the fetus, placenta and umbilical cord, and the blood loss at delivery and postpartum. The total need for elemental iron at this time is approximately 800 to 1000 µg (Hallberg, 1992).

The regulation of iron in the body is controlled mainly by absorption. When reserves are depleted there is a mechanism that enhances iron absorption by the intestinal cells. Once absorbed by the gut, iron is transported across the mucosal cells into the blood where it is added by a protein to bone marrow to join the production of erythrocytes. The primary cause of anemia during pregnancy worldwide is iron deficiency secondary to chronic inadequate dietary intake and menstruation,

heightened by the physiologic demands of the fetus and maternal blood volume expansion during pregnancy. Genetic causes and poor hygiene that may lead to infections and infestations are other contributing factors (Frewin *et al*, 1997).

1.2.2.3.2 Folate deficiency

Folate requirements are increased approximately twofold in pregnancy and serum folate levels fall to approximately half the normal range with a less dramatic fall in cell folate. In some parts of the world, megaloblastic anemia during pregnancy is common because of a combination of poor diet and exaggerated folate requirements. Given the protective effect of folate against neural tube defects, folic acid 400 microgram per day should be taken throughout pregnancy (Hoffbrand *et al*, 2006).

1.2.2.3.3 Vitamin B₁₂ deficiency

Vitamin B₁₂ deficiency is rare during pregnancy although serum vitamin B₁₂ levels fall to below normal in 20-30% of pregnancies and low values are sometimes the cause of diagnostic confusion (Hoffbrand *et al*, 2006).

1.2.2.2.4 Leukocytes and immune function

White blood cell count is increased in pregnancy. 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 and increase due to impaired neutrophilic apoptosis in 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. Lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester (Mello and Neme, 2000).

1.2.2.2.5 Haemostatic changes during pregnancy

During normal pregnancy there are changes of the vascular endothelium of the blood flow, anticoagulant factors and fibrinolysis. These changes begin from 10th week of gestation, coinciding with the rise of estrogen and progesterone, possible causal

elements. Such changes may reflect adaptive mechanisms, but can generate a hypercoagulable state, resulting in the development of thrombosis (Arruda and Souza 2002).

The platelet count typically falls by approximately 10% in an uncomplicated pregnancy. In approximately 7% of women this fall is more severe and can result in thrombocytopenia (platelet count $<140 \times 10^9/L$). In over 75% of cases this is mild and of unknown cause, a condition referred to as incidental thrombocytopenia of pregnancy. Approximately 21% of cases are secondary to a hypertensive disorder and 4% are associated with immunethrombocytopenic purpura. The platelet volume distribution width is raised and platelet aggregation 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 (Hoffbrand *et al*, 2006).

Pregnancy is associated with significant changes in the haemostatic profile. Fibrinogen and clotting factors VII, VIII, X, XII, vWF and ristocetin co-factor activity increase remarkably as gestation progresses. Increased levels of coagulation factors are due to increased protein synthesis mediated by the rising estrogen levels. Thus, pregnancy is a prothrombotic state. In pregnancy, aPTT is usually shortened, by up to 4 seconds in the third trimester, largely due to the hormonally influenced increase in factor VIII. However, no marked changes in PT or TT occur. There are changes in the levels and activity of the natural anticoagulants also. Levels and activity of Protein C do not change and remain within the same range as for non-pregnant women of similar age. Levels of total and free Protein S decrease progressively with the advancement of gestation. Antithrombin levels and activity are usually stable throughout the pregnancy. Acquired activated Protein C (APC) resistance has been found to occur in pregnancy, even when Factor V Leiden and antiphospholipid antibodies are not present. This has been attributed to the high factor VIII and factor V activity and low free Protein S levels (Arruda and Souza, 2002).

1.2.3 Complete blood cell count

A complete blood cell count (CBC) is a blood panel requested to give information about the cells in a patient's blood and identify illnesses such as infections, anemia, leukemia and other disorders of the blood.

A complete blood count will include:

White cells: Total white blood cells , the number of total white blood cells is given as absolute number per liter. All the white cell types are given as a percentage and as an absolute number per liter.

Red cells: Total red blood cells , the number of red cells is given as an absolute number per liter.

Hemoglobin: Hemoglobin, the amount of hemoglobin in the blood, expressed in grams per deciliter.

Hematocrit: Hematocrit or packed cell volume (PCV) this is the fraction of whole blood volume that consists of red blood cells.

Red blood cell indices :

- Mean corpuscular volume (MCV) is the average volume of the red cells, measured in femtolitres.
- Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin per red blood cell in picograms.
- Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in the cells.

Platelets: Platelet numbers are given, as well as information about their size and the range of sizes in the blood (Lewis *et al*, 2001).

1.2.3.1 Previous studies

Baig(2008): A high percentage of women in Pakistan at 20 to 26 weeks of pregnancy had mild to moderate anemia. Pica, tea consumption and low intake of eggs and red meat were associated with anemia.

Esmatet *al*, (2010) concluded that the prevalence of anemia in Iranian women during pregnancy is considerably lower than that of most countries or the one reported by WHO for Iran (> 40%) due to the improvements of the national health system and prenatal programs in recent years.

Haidar (2012) studied the prevalence and deficiencies of iron and folic acid in Ethiopian pregnant women and found anemia associated with illnesses (71.6%) was more prevalent than with deficiencies in iron (34%) and folic acid (24%).

Rania (2012) conducted at Port Sudan city, the results indicated that RBC, PCV and lymphocytes of Sudanese pregnant women attended Sea Ports corporation Hospital and Port Sudan Teaching Hospital decreased significantly while MCHC increased significantly compared to control. TWBC and neutrophils increased significantly in the third trimester compared to the first and second trimester.

Elgari (2013) found the most common type of anemia among sudanese pregnant women is microcytic hypochromic type and likely to be of iron deficiency, second class is normocytic normochromic type and less of occurrence type is dimorphic picture types. RBCs, PCV, Hb, lymphocytes and platelets were decreased significantly but TWBCs count was increased significantly specially neutrophils and insignificantly increased mixed count.

Patricket *al*,(2013)asses the hematological change during normal pregnancy in Nigeriaand concludedthathematological indices changes during normal pregnancy with significant difference of the test group when compared to the control , there were significant decrease in Hb, RBCs, PCV, MCV, MCH and MCHC. TWBCs count was increased significantly in contrast platelets count significantly lower than the normal control.

Mohamed (2015) studied the complete blood cell count of Sudanese pregnant women at the first trimester and the result indicated that MCHC decreased significantly. PCV and MCV increased significantly compared to control group. According to age group, history of abortion and number of pregnancies in pregnant women there were no significant difference between groups.

Albadri (2015) studied the complete blood cell count of Sudanese pregnant women at the second trimester and the result indicated that RBC, PCV, Hb, lymphocytes and platelets decreased significantly. TWBC and neutrophils increased significantly compared to control group. According to age group, history of abortion and number of pregnancies in pregnant women there were no significant difference between groups.

Mohamed (2015) assesses the complete blood cell count of Sudanese pregnant women in the third trimester attended Al-Rebat Teaching Hospital in Bahri State (Obstetric department) and the results indicated that RBC, PCV, Hb, lymphocytes and platelets decreased significantly while TWBC and neutrophils increased significantly compared to control group. According to age group, history of abortion and number of pregnancies in pregnant women there were no significant difference between groups.

1.3 Rationale

Normal pregnancy is often associated with physiological, hematological complications. Continuous assessment of the hematological profile of pregnancy may minimize complications.

Few published data are available in Sudan about hematological status of pregnancy and different trimesters, the result of this study could be baseline data in Sudan and so medical laboratories will provide pregnancy adjusted reference ranges which could help in diagnosis and management.

1.4 Objectives

1.4.1 General objectives

To determine CBC of normal Sudanese pregnant women at first trimester attended Al-Mokhtabar Medical Laboratory in Bahri locality.

1.4.2 Specific objectives

1. To determine the frequency of the age, number of pregnancies and history of abortion among the normal pregnant women.
2. To compare Hb, RBCs, PCV, MCV, MCH, MCHC, TWBCs, differential leukocyte count and platelet of normal pregnant women at first trimester and non pregnant women.
3. To compare CBC according to age, number of pregnancies of pregnant women.