بسم الله الرحمن الرحيم

Sudan University of Technology & Sciences

College of Graduate Studies

Assessment of Complete Blood Count of Sudanese Pregnant Women in the third Trimester in Khartoum State

تقيم تعداد الدم الكامل للنساء السودانيات الحوامل في الثلث الأخير من الحمل في ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc Degree in Hematology and Immunohematology

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الآيـــــــــــة

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قال تعالى :

﴿ وَقَضَىٰ رَبُّكَ أَلَّا تَعْبُدُوا إِلَّا إِيَّاهُ وَبِالْوَالِدَيْنِ إِحْسَانًا إِمَّا يَبْلُغَنَّ عِندَكَ الْكِبَرَ أَحَدُهُمَا أَوْ كِلَاهُمَا فَلَا تَقُل لَّهُمَا أُفٍّ وَلَا تَنْهَرْهُمَا وَقُل لَّهُمَا قَوْلًا كَرِيمًا ﴿ 23 ﴾

صدق الله العظيم

سورة الإسراء الاية رقم (23)

Dedication

To my parents

my brothers

my sisters

To everyone struggle to support me ....

Salma

Acknowledgements

I would like to thank my wonderful teacher, Dr. Khalda M.Hamza whose encourage, guidance and support me from the initial to the final level enabled me to develop this research.

To my truly great friends who have made available their support in a number of ways.

Lastly, special offer and blessings to all of those who supported me in any respect during the completion of the study.

Abstract

This is a case control study, conducting at Khartoum State the during period from February to June 2015. The aim of the study was to determine complete blood count (CBC) of Sudanese pregnant women in the third trimester attended Al-Rebat Teaching Hospital in Bahri State (Obstetric department).

Eighty healthy apparently pregnant women and forty non pregnant women were informed about the study and agreed for participation. A questionnaire was designed to collect information about the study group such as age, number of pregnancies, stage of trimesters and history of abortion. Three ml of venous blood was collected in EDTA anticoagulant container. Automated hematological analyzer (Sysmex KXN-21) was used to measure CBC.

The results indicated that RBC (mean 4.0×10^6/ml ± 0.4), HCT (mean 33.4% ± 3.0), Hbg (mean11.5 g/dl ± 1.3), platelets (mean 238.9 × 10^3/ml ± 55.0) decreased significantly (P-value 0.000, 0.000, 0.002, 0.000 respectively) while MCV (mean 83.7 fl ± 5.0), MCH (mean 28.9 pg ± 2.8) and MCHC (mean 34.5% ± 1.9) increased insignificantly. WBC (mean 6.9×10^3/ml ± 1.7) and neutrophils (mean 65.3% ± 8.3) increased significantly (P-value 0.009, 0.000) while lymphocytes (mean 25.1% ± 6.8) decreased 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 in spite of hemoglobin decrease by increase age and number of pregnancies.

المستخلص

هذه دراسة وصفيه تحليليه وصفيه أجريت في مدينه الخرطوم من الفترة من يناير إلي يونيو(2015) بهدف قياس نسب تعداد الدم الكامل للنساء في الثلث الأخير من فتره الحمل المترددات على مستشفى الرباط الجامعي في محليه بحري (قسم الولادة).

أخذت ثمانون عينه من نساء حوامل أصحاء و أربعون من غير الحوامل بعد إخطارهن عن الدراسة وموافقتهم على المشاركة. صمم استبيان لجمع معلومات عن المشتركات في الدراسة بالنسبة للعمر، عدد مرات الإنجاب، فتره الحمل و تاريخ وجود إجهاض. تم جمع 3مل دم في حاوية تحتوي على مانع تجلط (ثنائي أمين الايثيلين رباعي حمض الخل) من كل المشتركات. تم استخدام جهاز (سيسمكس9 لتحليل تعداد الدم الكامل والذي يعمل أوتوماتيكيا.

أشارت النتائج إلي إن كريات الدم الحمراء (متوسط = $4.0 \times 10^6 \pm 0.4$) ، الهيماتوكريت (متوسط= 3.0 ±% 33.4)، الهيموقلوبين (متوسط = 1.3 ± 11.5)، الصفائح الدموية (متوسط = 55.0 ± 238.9) انخفضت انخفاض ذو دلاله إحصائية (مستوى معنوية 0.000، 0.000، 0.002 على التوالي) في حين متوسط حجم الخلايا (متوسط = 5.0 ± 83.7) ومتوسط خضاب الدم في الخلايا (متوسط = 2.8 ± 28.9) ومتوسط تركيز خضاب الدم في الخلية (متوسط = 1.9 ± 34.5) تزيد زيادة ليست ذات دلالة إحصائية. كريات الم البيضاء (متوسط= $6.9 \times 10^3 \pm 1.7$) والخلايا المتعادلة (متوسط = 8.3 ±% 65.3) تزيد زيادة ذات دلالة إحصائية (مستوى معنوية 0.009، 0.000) بينما الخلايا الليمفاويا (متوسط = 6.8 ±% 25.1) تنخفض انخفاض ذات دلالة إحصائية مقارنه بالمجموعة الضابطة .

طبقا للعمر تاريخ وجود الإجهاض وعدد مرات الحمل في الناس الحوامل لا يوجد اختلاف في الدلالة الإحصائية بين المجموعات على الرغم من أن الهيموقلوبين يقل بزيادة العمر وعدد مرات الحمل.

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List of Abbreviation

CBC	Complete Blood Count
EDTA	Ethylene Diamin Tetra Acetic Acid
ESR	Erythrocytes Sedimentation Rate
Hb	Hemoglobin
HCT	Hematocrit
IDA	Iron Deficient Anemia
IL	Interleukins
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
PCV	Packed Cell Volume
PLTs	Platelets
RBCs	Red Blood Cells
RDW	Red cell distribution width
SCF	Stem cell factor
TWBCs	Total White Blood Cells
WHO	World Health Orgnization

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Chapter one

Introduction and literature review

Chapter one

INTRODUCTION AND LITRATURE REVIEW

1.1 Introduction

Pregnancy is often associated with physiological and haematological changes. This change may leads to complication during and after Pregnancy. In developing countries pregnant women didn't receive special medical care which may be due to socio-economicalstatus of most of Sudanese families didn't allow pregnant women to take supplement during pregnant period. In addition antenatal care clinic aren't available in all cites ,which difficult make regular checkup and the low (WHO, 2014).

Young adolescents face a higher risk of complications and death as a result of pregnancy than older women. About 16 million girls aged between 15 – 19 and some 1 million girls under 15 years give birth every year – most and middle income countries. Complications during pregnancy and childbirth are the second cause of death for 15-19 year old girls globally (WHO, 2014).

Skilled care before, during and after childbirth save the lives of women and newborn babies.Between 1990 and 2013, maternal mortality worldwide dropped by almost 50%.Maternal mortality is unacceptably high. About 800 women die from pregnancy and childbirth related complications around the world every day. In 2013, 289000 women died during and following pregnancy and childbirth. Almost all of these deaths occurred in low-resource settings, and most could have been prevented(WHO, 2014).

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

This study aimed to measure some of blood constituents that may be effected during pregnancy. Also the study include comparison between the measured

parameter according to age, number of pregnancies and history of abortion in pregnant women at the third trimester compared to non-pregnant women attended to Al-Rebat Teaching Hospital in Bahri State (Obstetric department).

1.2 Literature Review

1.2.1 Blood

1.2.1.1 Blood Function

Blood is a fundamental component of life. Within the adult body approximately 4 to 5 liters of blood circulates continuously through vessels. As its moves from lungs and heart feed the cells by nutrients include oxygen which is most basic elements necessary for survival. Also blood picks up wastes such as carbon dioxide, that ultimately be removed from the body as the blood back to lungs (Rogers, 2011).

1.2.1.2Blood composition

Blood composed of cells carried in plasma these cells are erythrocytes which transport oxygen, leukocytes act as immune defense and platelets for blood clotting. Plasma contain protein and water.PlasmaContains 3 types of proteins: albumin, globulins and fibrinogen. Use in maintain osmotic pressure, immune defense and blood clotting respectively (Fox, 2006).

All 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-cells and B-cells (types of white blood cells). This is lymphopoiesis.
- Myelocytes, which include granulocytes, megakaryocytes and macrophages and are derived from common myeloid progenitors, are involved in such diverse roles as innate immunity, adaptive immunity, and blood clotting. This is myelopoiesis.

Granulopoiesis (or granulocytopoiesis) is haematopoiesis of granulocytes.

Megakaryocytopoiesisis haematopoiesis of megakaryocytes(Fischbach and Dunning, 2009).

In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation of lymphoid cells occurs in secondary lymphoid organs (spleen, thymus, and lymph nodes). In children, haematopoiesis occurs in the marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum.

In some cases, the liver, thymus, and spleen may resume their haematopoietic function, if necessary. This is called extramedullaryhaematopoiesis. It may cause these organs to increase in size substantially. During fetal development, since bones and thus the bone marrow develop later, the liver functions as the main haematopoetic organ. Therefore, the liver is enlarged during development (Hoffbrand, 2006).

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 (Fischbach and Dunning, 2009).

Haemopoiesis need growth factors to regulate proliferation, differentiation, maturation of blood cells and prevent apoptosis, this growth factors are glycoproteins hormones which may act locally at site of cell production or

circulate in plasma. Depend on this growth factors and certain cytokines cell may divide to lymphoid and myeloid.

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 haematopoietics 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 CSF (GM-CSF), granulocyte CSF (G-CSF) and macrophage CSF (M-CSF). These stimulate granulocyte formation and are active on either progenitor cells or end product cells (Edward *et.al*, 2005).

Erythropoietin is required for a myeloid progenitor cell to become an erythrocyte. On the other hand, thrombopoietin makes myeloid progenitor cells differentiate to megakaryocytes (thrombocyte-forming cells). Examples of cytokines and the blood cells they give rise to.

Myeloid progenitor For a decade now, the evidence is growing that haemopoitic stem cell (HSC) maturation follows a myeloid-based model instead of the 'classical' schoolbook dichotomy model. In the latter model, the HSC first generates a common myeloid-erythroid progenitor (CMEP) and a common lymphoid progenitor (CLP). The CLP produces only T or B cells. The myeloid-based model postulates that HSCs first diverge into the CMEP and a common myelo-lymphoid progenitor (CMLP), which generates T and B cell progenitors through a bipotential myeloid-T progenitor and a myeloid-B progenitor stage. The main difference is that in this new model, all erythroid, T and B lineage branches retain the potential to generate myeloid cells (even after the segregation of T and B cell lineages). The model proposes the idea of erythroid, T and B cells as specialized types of a

prototypic myeloid haemopoitic stem cell (HSC) (Fischbach and Dunning, 2009).

Myeloid precursors differentiate further into the erythrocytes, granulocytes and thrombocytes lineages to drived red cell, granulocytes, monocytes and platelets to circulation. Cell production is highly controlled through cytokines and humoral loops which can be increased rapidly in response to demand.

Lymphoid precursors which produce in two stage primary lymphoid organ in bone marrow and thymus in which lymphocytes develop. Secondary lymphoid organ in which specific immune responses are generated are lymph nodes, spleen and lymphoid tissues of alimentary and respiratory tracts.

Erythropoiesis is production of red cell by number of cell divisions from pronormoblasts which is large cell with dark blue cytoplasm, central nucleusandslight clump of chromatin to normoblast they progressively more haemoglobin which stain pink in cytoplasm. Cytoplasm stain paler blue as it lose it's RNA while chromatin becomes more condensed.

Finally nucleus extruded from late normoblastin the marrow and produce reticulocytes which still contains some ribosomal RNA and still able tosynthesize haemoglobin and spend 1-2 days in marrow and also circulates in peripheral 1-2 days completely lose RNA result non-nucleated biconcave disc (Lewis *et.al*, 2001).

Red cell haemoglobin and anaemia is reduced amounts of Hb accompany an overall reductionin body iron in iron deficiency anaemia or acute bloodloss in other anaemias such as the megaloblasticanaemia, iron redistributed from red cells tomarrow stainable iron and serum ferritin level (Hoffbrand, 2006). Anaemia is considered when Hb is less than 11g/dl forchildren aged 0-4years and pregnant women, Hb is lessthan 12g/dl for children aged 5-12 years and non pregnant women and Hb is less than 13g/dl for men (William, 2002).

1.2.2 Pregnancy

Normal pregnancy involves many changes as normal adaptations that a woman undergoes during pregnancy to better accommodate the embryo or fetus.

1.2.2.1 Physiological changes during pregnancy

Physiological changes that are entirely normal include 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 to ensure the fetus is provided for, to ensure fetus grows properly and receives adequate nutrition. Increase in blood sugar, breathing and cardiac output are all required. Level of progesterone and estrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle.Prolactin level also increase due to maternal pituitary gland enlargement by 50%. In addition to Parathyroid hormone is increased which leads to increases of calcium uptake in the gut and reabsorption by the kidney. Adrenal hormones such as cortisol and aldestrone are also increase. Women and the placenta also produce many hormones such as Human placental lactogen (hpl) and stimulates lipolysis and fatty acid metabolism by woman, conserving blood glucose for use by the fetus, it can also decrease maternal tissue sensitivity to insulin, resulting in gestational diabetes.These changes include expansion in maternal blood and plasma volume. Plasma volume begin to increase within a few weeks after conception and reaches a maximum at approximately 34 weeks (Brown *et.al*, 2011).

1.2.2.2 Hematologicalchanges during pregnancy

Hematologic change includeincrease in plasma volume which is relatively larger than the increase in red cell mass resulting in a relative anemia.This results in a physiologically lowered hemoglobin (Hb) level, hematocrit (HCT) value, and RBC count, but it has no effect on the mean corpuscular

volume (MCV). An increase in the levels of some plasma proteins alters the balance of coagulation and fibrinolysis. Worldwide, the predominant cause of anemia in pregnancy is iron deficiency. Bleeding disorders in pregnancy are a common reason for hematologic consultation and evoke concern for both the mother and child. Life-threatening bleeding caused by disseminated intravascular coagulation is seen with some complications unique to pregnancy, including placental abruption, retained dead fetus, and amniotic fluid embolism and many others diseases may involve(Fischbach and Dunning, 2009).

Maternal blood volume increases by an average of 40 to 50 percent above the nonpregnantlevel. Plasma volume begins to rise early in pregnancy, with most of the escalation taking place in the second trimester and prior to week 32 of gestation. Red cell mass increases significantly beginning in the second trimester and continues to expand throughout pregnancy, but to a lesser extent than plasma volume. Erythropoietin levels increase throughout pregnancy, reaching approximately 150 percent of their prepregnancy levels at term. The overall effect of these changes in most women is a slight drop in hemoglobin concentration, which is most pronounced at the end of the second trimester and slowly improves approaching term(Fischbach and Dunning, 2009).

1.2.2.3 Types of Anemia during pregnancy :-

The simplest approach to the differential diagnoses of anemia is to differentiate anemias by the mean corpuscular volume (MCV), measured in fL.

MCV less than 80 fL or microcytic anemia etiologies are as follows:

- Iron deficiency
- Thalassemia
- Anemia of chronic disease
- Sideroblastic anemia

- Anemia associated with copper deficiency
- Anemia associated with lead poisoning

MCV 80-100 fL or normocytic anemia etiologies are as follows:

- Hemorrhagic anemia
- Early iron deficiency anemia
- Anemia of chronic disease
- Anemia associated with bone marrow suppression
- Anemia associated with chronic renal insufficiency
- Anemia associated with endocrine dysfunction
- Autoimmune hemolytic anemia
- Anemia associated with hypothyroidism or hypopituitarism
- Hereditary spherocytosis
- Hemolytic anemia associated with paroxysmal nocturnal hemoglobinuria

MCV greater than 100 fL or macrocytic anemia etiologies are as follows:

- Folic acid deficiency anemia
- Vitamin B-12–deficiency anemia
- Drug-induced hemolytic anemia (eg, zidovudine)
- Anemia associated with reticulocytosis
- Anemia associated with liver disease
- Anemia associated with ethanol abuse
- Anemia associated with acute myelodysplastic syndrome

1.2.2.3.1 Iron deficiency anaemia

due to up to 600mg required for increase in red cell mass and 300mg for fetus. In uncomplicated pregnancy MCV rises about 4fl. Fall in MCV is earliest sign of iron deficiency. Later MCH falls and finally anaemia results. Early iron deficiency if serum ferritin is below 15microg/L and serum iron < 10mircomol/L which should treat with iron supplements. Routine iron

supplements better avoid until Hb below 10g/dl or MCV below 82fl in third trimester (Hoffbrand, 2006).

When iron utilization is increased in infancy and during growth, or when concurrent blood loss occurs, as in menstruation, dietary intake may be insufficient, especially because women and children tend to consume less than the recommended minimal daily requirement. The problem increases during pregnancy, when some iron is diverted to the fetus for hematopoiesis, and with breast feeding, when iron is lost in the milk (Douglas and Hirschmann, 2007).

- **Prevalence of iron deficiency anemia world wide:**

Iron deficiency anemia is serious public health problem effecting more than 700 million people in world. (Dawood*et.al*,1990) Its more in developing regions (59%) than in industrialized world (14%). (DeMaeyer, 1989) Previous studies on iron deficiency anemia prevalence 39.7% in Kuwait(Dawood*et.al*,1990), 78% in Liberia(Jackson, 1982), 73.9% in Jamaica (Simmon*et.al*, 1982), 50% in Bahrain(Aldallal, 1984), 21.1% in Egypt(National nutrition survey,1978) and 19.8% in Northern Irelland(Strain *et.al,*1990).

Data presented in table 1.1 below shows worldwide prevalence rate of IDA(Duran, 2000)

Table 1.1 worldwide prevalence rate of IDA(Duran, 2000)

Regions	Population with ID (millions)	Prevalence of anemia i pregnant women(%)
Africa	206	52
Americas	94	40
Europe	27	18
Eastern Mediterranean	149	50
Southeast pacific	616	74
Western pacific	1058	40
developed countries		18
developing countries		56
Total	2150	51

1.2.2.3.2 Folate deficiency

Folic acid deficiencyis much less common than iron deficiency. It's occur when the body's demand for it increases, as in pregnancy in which requirements increased twofold and conditions associated with increased cell turnover, such as acute exacerbations of hemolytic anemia, leukemia, and exfoliative dermatitis. Some medications, such as methotrexate and trimethoprim, cause folate deficiency by altering its metabolism (Douglas and Hirschmann,2007).

An increased MCV (typically >100 fL) can be suggestive of folate and/or B-12 vitamin deficiency; in this case, determine serum levels of vitamin B-12 and folate. .So megaloblasticanaemia during pregnancy is commonly with poor diet and increase Folate requirements. To protect against neural tube defects folic acid 400microg/day should taken throughout pregnancy.

1.2.2.3.3 Vitamin B_{12} deficiency

rare during pregnancy although serum vitamin B12 level fall below 20-30% of normal and low value cause of diagnostic confusion.

Sometimes, pure red cell aplasia occurs during pregnancy without any apparent explanation and typically disappears following delivery (Douglas and Hirschmann,2007).

1.2.2.4 Platelets

The effect of pregnancy on maternal platelet count is somewhat more controversial; some studies demonstrate a mild decline in platelet count over the course of gestation, Commonly approximately 7% of pregnancies. Its typically defined as platelet count lower than150.000/ml. the most common cause of thrombocytopenia during pregnancy is gestational thrombocytopenia, which is mild thrombocytopenia with platelet levels remaining above 70.000/ml. patient who are effected usually are asymptomatic and have no history of thrombocytopenia before pregnancy. Their platelet should retain to normal within several weeks following delivery(WHO,2014).

Normal pregnancy is characterized by an increase in platelets aggregation and decreased their number in circulation with gestation. Platelets lifespan decline during pregnancy. Increased consumption of platelets in uteroplacental circulation has been explanation of the reduction in the number (Juan*et.al,* 2011).

1.2.2.5 White Cell Counts

White cell counts rise during pregnancy with the occasional appearance of myelocytes or metamyelocytes in the blood. During labor and the early puerperium, there is a rise in the leukocyte count. Leukocytosis appears to be linearly related to the duration of labor(Kenneth *et.al*, 2010).

1.2.2.6 Plasma Proteins

The levels of some plasma proteins also increase during pregnancy. In particular, C-reactive protein concentration is higher in pregnant women and rises even further during labor. Erythrocyte sedimentation rate (ESR) rises during pregnancy, and is affected by both hemoglobin concentration and gestational age. The rise in ESR during pregnancy, in large part a result of an increase in levels of plasma globulins and fibrinogen, makes its use as a marker of inflammation difficult. The levels of many of the procoagulant factors increase during pregnancy whereas activity of the fibrinolytic system diminishes in preparation for the hemostatic challenge of delivery(Fox, 2006).

1.2.3 Complete Blood Count :-

Complete blood count (CBC) gives important information about the kinds and number of cells in the blood, especially red blood cells, white blood cells and platelets. CBC helps check any symptoms, such as weakness, fatigue or bruising you may have. such as anemia, infection and many other disorders.

Mohamad(2007)studied iron deficiency among pregnant women who attend to antenatal care center in Nablus she found that most of whom had IDA were in third trimester.

Tiwar(2012) found that iron deficiency anaemia (IDA) is the most common cause of anaemia in pregnancy in Indians and is associated with increased risk of low birth-weight infants. Studies from developed countries recommend iron supplementation based on serum ferritin levels.

Akinbam(2013)in Lagos Nigeria: Anemia is the most common hematological problem in pregnancy ,followed by thrombocytopenia. Leukocytosis is almost associated with pregnancy. They were study 274 pregnant women who were attending to clinic between their first and third trimesters. He found a statistically significant relationship was found to exist between PCV and WBC count with increase in gestational age. However there was no

statistically significant association between platelet count and increase in gestional age.

Abd-Elsalam(2012)assessed CBC in pregnant women in Port Sudan and the study is cross-sectional case control descriptive analytical study to determine Hb, RBCs, HCT, MCV, MCH, MCHC, Platelets, WBCs, RDW, MPV and differential count of Sudanese pregnant women and they were collect information about age, number of pregnancy, stage of trimester and history of abortion by automated hematological analyzer (sysmex KXN-21) any they found compared to control WBC and neutrophils significantly increased. Hemoglobin, Platelets, eosinophils, basophils ,MPV insignificantly decreased while MCV, MCH and RDW increased insignificantly.

Elgari(2013) found there were significant decreased in RBCs count, hemoglobin (Hb) and packed cell volume (PCV) of Sudanese pregnant women compared to non pregnant women and significant decreased in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pregnant women .TWBCs count was increased significantly in contrast platelets count significantly lower than the normal control.

Abdalla(2014) they were found that iron deficiency anaemia is the most common nutritional deficiency in the world. Because of the increased iron requirements of pregnancy and growth, pregnant women and infants are recognized as the groups most vulnerable to iron deficiency anaemia.

1.3 Rationale

Pregnancy in developing countries have high mortality ratio and higher risk specially young adolescents who face higher risk of complications of death as a result of pregnancy than older women (WHO, 2014).

Women in developing countries didn't receive enough care due to socio-economic causes and they couldn't receive special care. Most of Sudanese families couldn't afford to provide pregnant women to take supplement during pregnancy. In addition antenatal care clinic aren't available in all cites which difficult make regular checkup.

Few studies were available about hematological profile of Sudanese pregnant women. This research is base line data to formulate clinical files about hematological status of Sudanese Pregnant women.

1.4 Objectives

1.4.1 General objective

Measurment of some hematological parametersin Sudanese normal pregnant women at the third trimester.

1.4.2Specific objective

1 / TomeasureHb, RBCs, HCT, MCV, MCH, MCHC, RDW, WBCs, diferential leukocyte count and Platelets of pregnant women at the third trimester compare to control gruop.

2 / Todetecteffect ofage group, number of pregnancy and history of abortion inRBC, HGB, HCT, TWBCS and PLTS.

Chapter two

MaterialsandMethods

Chapter Two

Materials and Methods

2.1Study design

Case control study conducted during period from February to June(2015).

2.2 Sample size

The study included 120 samples.Include eighty (80) pregnantwomen at third trimester and forty (40) nonpregnant women in same age group.

2.3Study area

Sample conducted in Khartoum north in Bahri state at Al-Rebat Teaching Hospital

2.4Study population

Sudanese pregnant women in third trimester attended toAl-Rebat hospital in Bahre state (Khartoum north-compass) and control group in same age group

2.4.1 Inclusion Criteria

Sudanese Pregnant womenat the third trimester with age group ranged from 18-41years.

2.4.2 Exclusion Criteria

Pregnant women at first or second trimesters.Pregnant women at third trimester such as Malaria , Typhoid fever, previous history of anemia or during menstrical cyle were excluded.

2.4.3 Sampling technique

Non Probability Voluntary sampling.

2.5 Method of data collection

A questioner was used to obtain demographic and clinic data includingage, number of pregnancies, stage of trimesters, use of supplement, history of abortion, present of disease during pregnancy and whether they regally visit clinics.

2.6 CBC determination

Full automated cell counter "Sysmex KX N21" .

2.7 Blood test and procedure

2.7.1 Specimen collection

3ml venous blood was collected in EDTA containerfrom all participants and complete blood count (CBC) was collected for third trimester pregnant women and non pregnant women (control).

Well and genital mix of sample before test. Examine within 2hrs at room temperature37^{o}C .

Take was care about medications which effect platelets like steroid, chemotherapy, some antibiotics, quinidine, merprobamate and thiazide diuretics.

Clotted sample wasavoid.

2.7.2 Requirements :

1. Automated Hematological analyzer SysmexKX21N .
2. EDTA containers (vacotainer).
3. Cotton, Blister and Tourniquet.

2.8 Method

2.8.1 CBC measurement

Counts were perform by Sysmex automated analyzer. Principal is based on the electronic resistance (impedance) detection and recognition of cell depend on size.Through using three preliminary hydrauic systems for WBCs, RBCs, platelets and hemoglobin and display the cell on (LCD) liquid crystal displayer with histogram. It perform 18 parameters and print paper also can bind to LIS system (Barbara, 2003).

2.8.2 Principle of Sysmex KX 21 N:

Diluted sample delivered to RBCs and platelets bath through aperture and counts. Other one into WBCs bath in which hemolytic reagent was added (stromatolyzer) to break RBCs and hemoglobin released and measured bycolorimetery based on cyanomethemoglobin method (HICN) by spectrophotometer absorption at 540nm. Cell in apertures produce pulses which convert to digital number programmed and designed for RBCs and WBCs depend on size. Red cell indices and differential and absolute calculated from given information. HCT was measured by integration volume of RBCs (Lewis*et.al*, 2006).

2.9 Quality Control (QC)

Known sample was examine by two others labs in Alsalam Hospital and Shafi Specialized Oncology Center.

2.10 Ethical consideration

Before collecting the sample the aim of study was explained to each participant. A written consent from legal and verbal from illegal was obtained before sample collection.Was used an ideal blood collection procedure in order to safe them.

2.11 Statistical analysis

Statistical Package of Social Sciences (SPSS) software program was used for statistical analysis one way ANOVA and Independent T-test were usedto obtain P-value significant level was set at ≤ 0.05 .

Chapter three

RESULTS

Chapter Three

Results

This is case control study conducted in Antenatal Clinic of Al-RebatHospital in Bahri state. Eighty (80) pregnant at the third trimester compare to forty (40) non-pregnant women all were between 18 – 41 years.

Complete Blood Count of pregnant women and non- pregnant women (control). showed that Hb, RBCs and HCT significantly decreased. MCH, MCV and MCHC increased insignificantly. Platelets also decreased significantly while WBCs increased significantly specially Neutrophils but lymphocytes significantly decreased as show in Table (3-1).

Complete Blood Count in pregnant women according to age group as show below in table (3-2) RBC, Hb and HCT were decreased insignificantly. MCH and MCV are decreased insignificantly in age groups ranged between (32-36) and (37-41) years while MCHC insignificantly decreased in only in age groups ranged (37-41)years.Platelets decreased insignificantly while WBCs increased .

In table (3-3) show no significant difference in CBC of between group of pregnant with different number of pregnancies.While RBC, Hb, HCT and platelets decreased insignificantly and WBC increased insignificantly. MCH, MCV and MCHC is insignificantly decreased in the group with (4-8) pregnancies.

A demographical data about age of pregnant women in third trimester showed in figure (3.1). And demographical data about frequently of abortion in figure (3.2) .

Table (3.1) Complete Blood Countofpregnant women in third trimester and control

Study group	Case n=80	Control n=40	P- value
Parameters	Mean ± SD	Mean ± SD	
RBC × 10^6 / ml	4.0 ± 0.4	4.4 ± 0.6	0.000
Hbg g/dl	11.5 ± 1.3	12.5 ± 1.6	0.002
HCT %	33.4 ± 3.0	36.6 ± 4.1	0.000
MCV fl	83.7 ± 5.0	83.2 ± 6.5	0.70
MCH pg	28.9 ± 2.8	28.4 ± 2.8	0.30
MCHC %	34.5 ± 1.9	34.1 ± 1.6	0.20
Plt × 10^3/ ml	238.9±55.0	321.3±91.5	0.000
WBC × 10^3/ ml	6.9 ± 1.7	6.0 ± 1.8	0.009
Neutrophil %	65.3 ± 8.3	55.8 ± 11.4	0.000
Lymphocytes %	25.1 ± 6.8	35.6 ± 10.6	0.000
Mixed %	9.6 ± 3.4	8.6 ± 3.8	0.15

Table (3.2) Complete Blood Countofpregnant women in third trimester according to age

Study group	I n = 16	II n = 29	III n = 23	IV n = 8	V n = 4	P-value
Parameters	Mean ±SD	Mean ± SD	Mean ±SD	Mean ±SD	Mean ±SD	
RBC × 10^6 / ml	4.0±0.5	4.0±0.3	4.0±0.4	4.0±0.5	4.0±0.1	0.98
Hb g/dl	11.4±1.5	11.8±1.0	11.8±1.1	10.6±1.6	10.6±0.4	0.06
HCT %	33.1±4.1	33.9±2.1	33.9±3.1	32.1±2.9	31.7±1.6	0.38
MCV fl	83.4±5.2	83.9±4.1	85.0±4.8	81.1±7.0	79.2±3.2	0.10
MCH pg	28.7±2.5	29.2±2.5	29.7±2.6	26.7±3.6	27.0±1.1	0.52
MCHC %	34.4±1.6	34.7±1.9	34.9±1.7	32.8±2.5	34.2±1.6	0.09
Plt × 10^3/ml	245.2±50.3	239.0±53.0	230.2±65.8	249.8±35.2	248.8±60.7	0.85
WBC × 10^3/ ml	7.1±1.7	6.7±2.0	7.2±1.6	7.0±1.3	6.6±1.0	0.86
Neutrophil %	66.1±7.4	63.2±8.9	66.8±8.5	65.2±6.8	69.0±3.4	0.47
Lymphocytes %	24.2±4.9	26.8±8.0	23.7±6.4	25.6±7.5	23.8±4.1	0.51
Mixed %	9.7±4.0	9.9±3.2	9.5±3.7	9.2±2.8	7.3±1.3	0.69

I= (17 –21) year , II= (22 -26) year, III= (27 -31) year, IV= (32-36) year, V= (37-41) year

Table (3.3) Complete Blood Countofpregnant women in third trimester according to number of pregnancies

Study group	I n = 17	II n = 46	III n = 17	P-value
Parameters	Mean ± SD	Mean ± SD	Mean ± SD	
RBC × 10^6 / ml	4.1 ± 0.3	4.0 ± 0.4	4.1 ± 0.3	0.52
Hb g/dl	12.0 ± 0.4	11.5 ± 1.4	11.1 ±1.1	0.14
HCT %	34.5 ± 1.8	33.2 ± 3.4	33.1 ± 2.5	0.16
MCV fl	84.9 ± 3.6	84.1 ± 5.5	81.2 ± 4.0	0.31
MCH pg	29.7 ± 2.3	29.2 ± 2.9	27.5 ± 2.3	0.26
MCHC %	34.9 ± 2.0	34.6 ± 1.8	33.8 ± 2.0	0.24
Plt × 10^3/ ml	239.7 ± 45.8	244.5 ± 62.4	222.9 ± 39.0	0.15
WBC × 10^3/ ml	6.9 ± 1.7	7.2 ± 1.9	6.3 ± 1.1	0.82
Neutrophil %	66.2 ± 6.9	65.2 ± 8.7	64.8 ± 8.3	0.99
Lymphocytes %	23.7 ± 5.8	25.5 ± 7.5	25.5 ± 5.8	0.89
Mixed %	10.1 ± 3.0	9.4 ± 3.7	9.6 ± 3.1	0.96

I = No pregnancy before, II = (1-3) pregnancies, III = (4-8) pregnancies

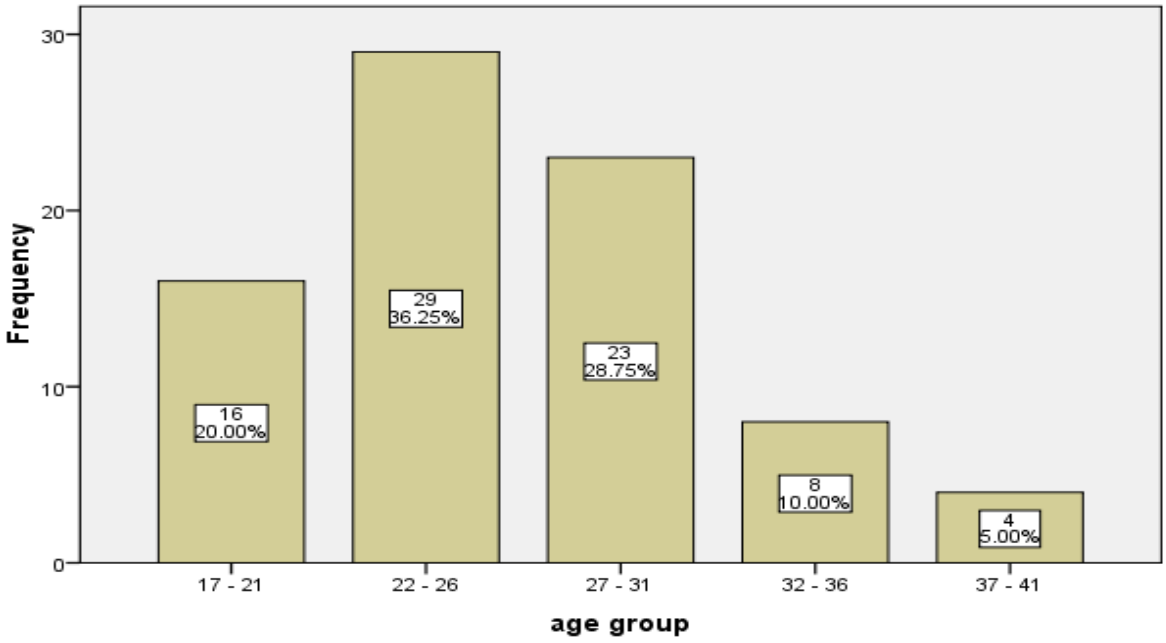

Fig (3.1) Distribution of Pregnant women according to age

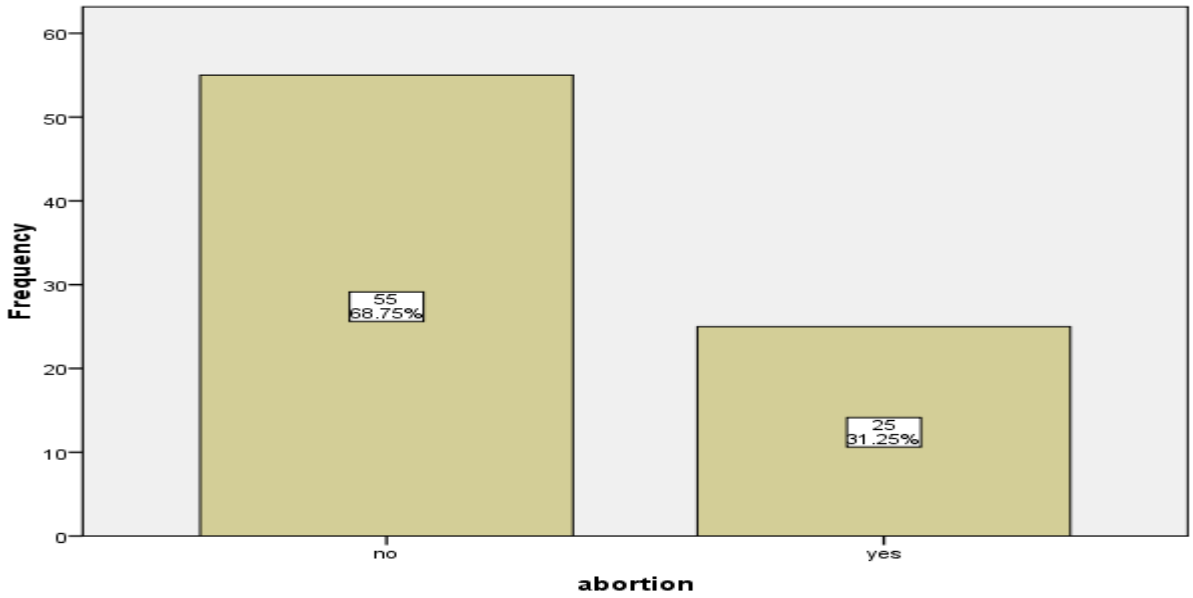

Fig (3.2) Distribution of Pregnant women according to history of abortion

Chapter four

Discussion, Conclusion and Recommendations

Chapter four

Discussion, Conclusion and Recommendations

4.1 Discussion

This study was carried out at Khartoum State during the period from February to June to determine the Hb, HCT, RBC, MCV, MCH, MCHC, WBC, Neutrophils, Lymphocytes, Mixed and Platelets of pregnant women attending Al-RebatTeaching Hospital in Bahri locality.Hb decreased significantly in pregnant women in third trimester compared to control. Similar finding obtained by (Elgari,2013) which indicated that Hb in pregnant women decreased compared to control . Also there were significantly decreased in RBC and HCT (P-value 0.000 and 0.000 respectively)similar to (Abd-Elsalam,2012) and (Elgari, 2013).

There no statistical significant different between case and control in MCV and MCH similar result obtain by (Abd-Elsalam,2012) while differ in MCHC which she had significant increased. While(Abdalla, 2014) obtained that only 2.5% (2 of 80) had low MCHC.

When iron utilization is increased in infancy and during growth, or when concurrent blood loss occurs, as in menstruation, dietary intake may be insufficient, especially because women and children tend to consume less than the recommended minimal daily requirement. The problem increases during pregnancy, when some iron is diverted to the fetus for hematopoiesis, and with breast feeding, when iron is lost in the milk. (Douglas and Hirschmann, 2007)this explain that way Hb was effected in pregnancies.

Platelets of pregnant women decrease significantly similar finding by (Elgari,2013).Normal pregnancy is characterized by an increase in plateletsaggregation and decreased their number in circulation with gestation. Platelets lifespan decline during pregnancy. Increased consumption of

platelets in uteroplacental circulation has been explanation of the reduction in the number (Juan*et.al,* 2011).

TWBC and Neutrophils of pregnant women increased significantly compared to control but lymphocytes significantlydecrease. A study done by(Abd-Elsalam, 2012) and (Elgari,2013) showed similar finding.

This increased may be as result of the body building the immunity of fetus and achieved by a state of selective immune tolerance, in the presence of strong antimicrobial immunity(Elgari,2013).

According to age of pregnant women there no significant different in spite of there were decreased in RBC, Hb, HCT, MCV, MCH, MCHC and Platelets insignificantly in group ranged between (32-36) years while WBC decreased insignificantly. similar result obtained by (Abd-Elsalam,2012).

In number of pregnancies there no significant different between groups in group of pregnancies between (4-8) show more decreases but still insignificantly similar (Abd-Elsalam, 2012).

From two last points that are confounding factor if decreased results from ages or number of pregnancies.

Demographic data show frequency of age in pregnant women in which nearly half of them between 17-26 years.

Also demographic data show frequency of history of abortion in pregnant women which about one third of them had previous abortion. From questionnaire it was due to of lack of knowledge and bad habits.

4.2 Conclusion

1. Hb, HCT and RBC of pregnant women in third trimester decreased significantly compared to control.
2. Hb and HCT insignificantly decreased in increase number of pregnancyand age increase .
3. MCV, MCH and MCHC of pregnant women in third trimesterdecreased insignificantly compared to control.
4. Platelets decreased significantly compared to control and decreased insignificantly with number of pregnant between 4-8.
5. TWBC and Neutrophils of pregnant women increased significantly compared to control. While Lymphocytes decreased significantly.
6. Most common type of anemia was normocytic normochromic anemia.

4.3 Recommendation

1. Daily oral iron and folic acid supplementation is recommended as part of the antenatal care to reduce the risk of maternal anemia and iron deficiency anemia.
2. Iron-rich diet and good dietary habits are cornerstones for prevention and treatment of IDA.
3. Follow up should be within minimum cost and to be available in different areas in Sudan.
4. Iron status (serum ferritin and serum transferrin receptor concentration)should usedfor best laboratory evaluation tests for IDA.
5. Complete CBC should be performed monthly during pregnancy.

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Appendices

Appendices (1)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Questionnaire to measure CBC of pregnant women in third trimester attended in Bahri locality

NO( )

Personal data:

Name : ……Age : …….

Residence : …….

Months of pregnancy : ……Number of pregnancy : ……

Abortion : Yes ( ) No ( )If yes, how many times : ……

Supplementation intake : Yes regular ( ) Yes irregular ( ) No ( )

Visit to clinic : Yes ( ) No ( )

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

Other : ……..

Previous blood transfusion : Yes ( ) No ( )If yes, when : ……

Result :-

RBCs …….WBC …….

HGB ……..…. NEUT % ……

HCT ……...MXD % ……..

MCV ……. LYMPH % …….

MCH ……..….NEUT # ……..

MCHC …….. MXD # ……

LYMPH # ……..PLT ……

Appendices (2)

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كلية الدراسات العليا - برنامج الماجستير – مختبرات طبية

تخصص علم أمراض الدم ومبحث المناعة الدموية

براءة أخلاقية

الاسم :.....

سوف يتم اخذ من الدم (3مل) من الوريد كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية

وأنا اقر بان هذه العينات يتم فقط تحليلها لغرض البحث

أوافق أنا المذكور أعلاه اخذ عينة لإجراء الدراسة

الإمضاء:.....

التاريخ:.....

Appendices (3)

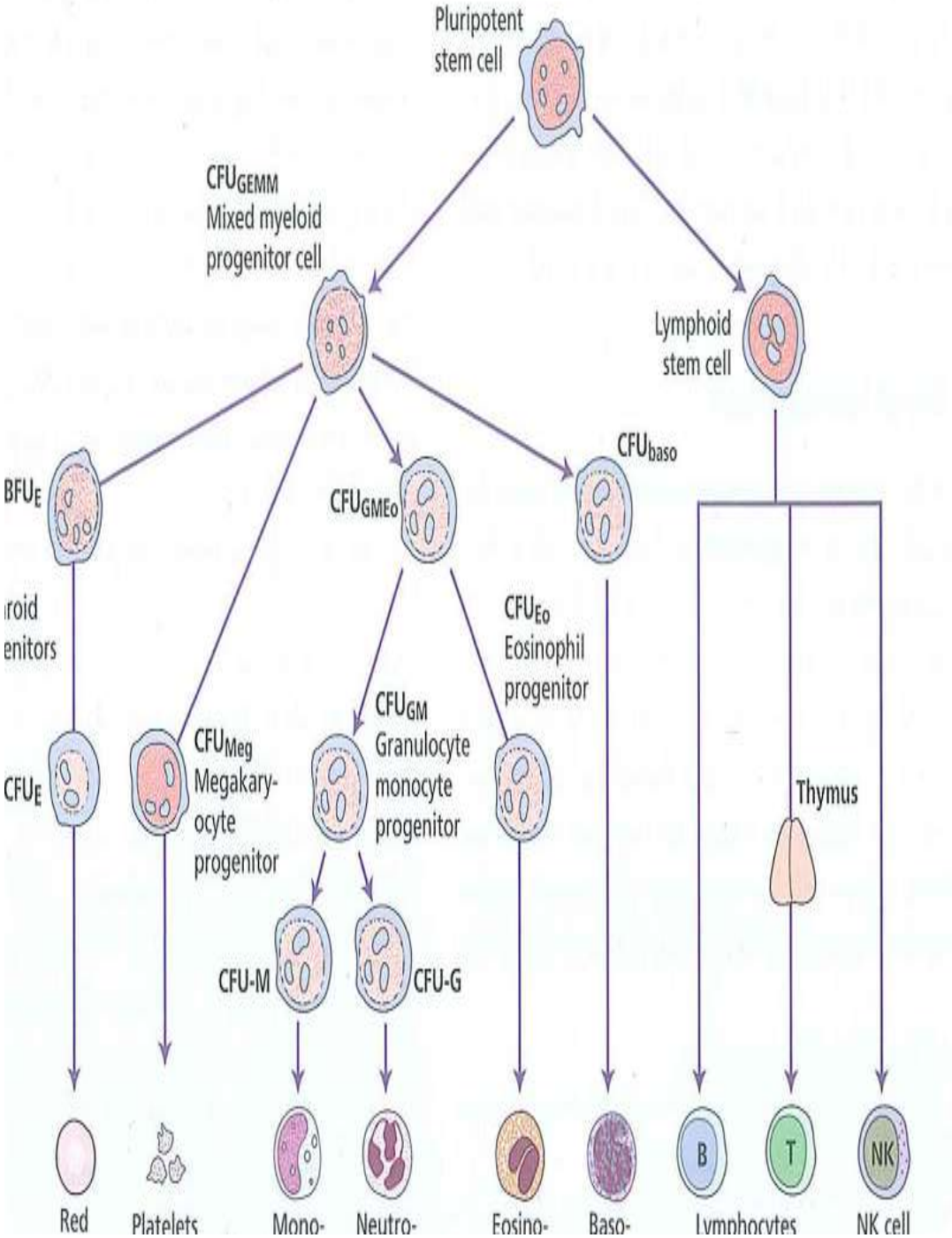

Figure (1) Pluripotent stem cell and the cell line arise from it.(Hoffbrand, 2006)

Appendices (4)

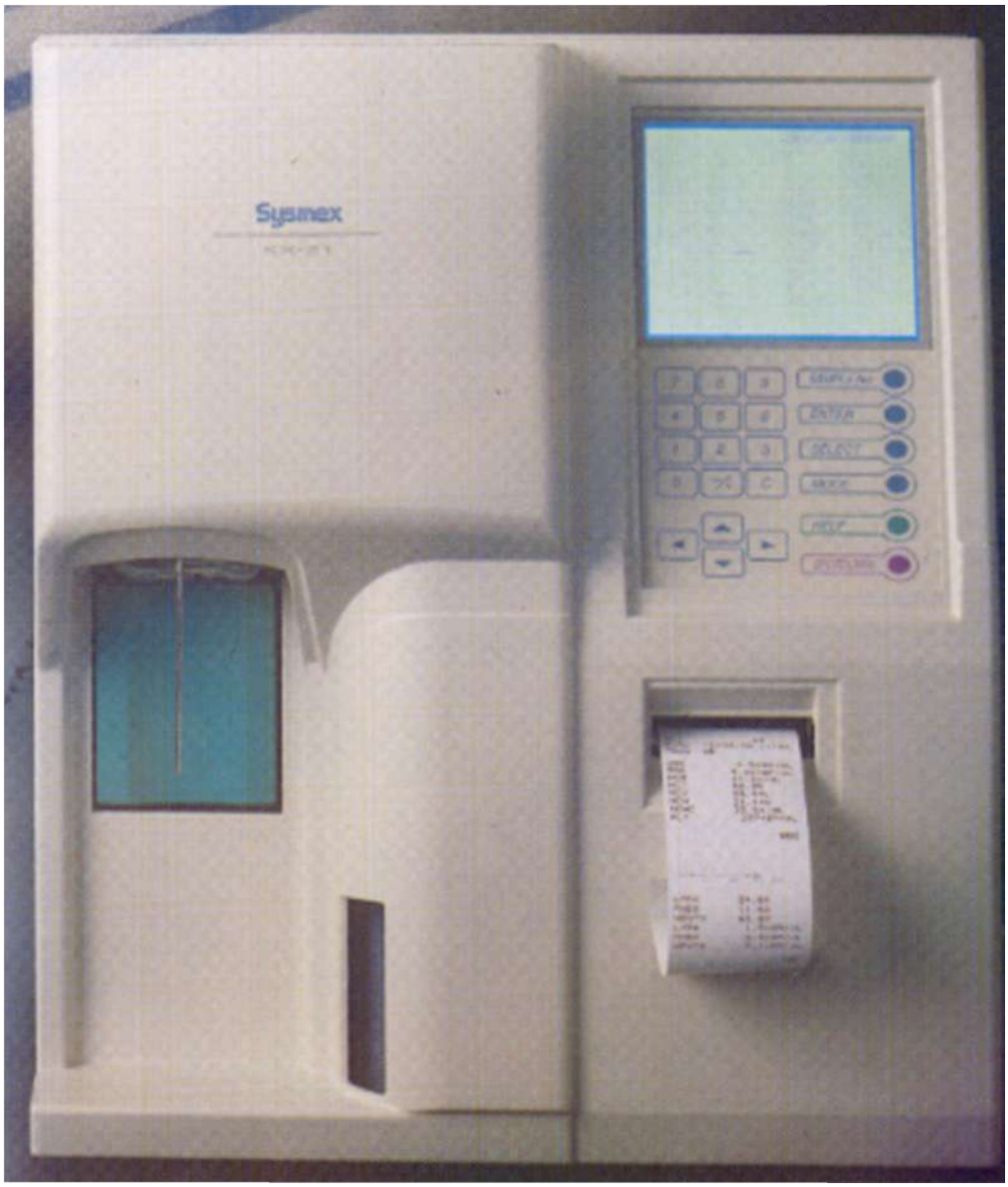

Figure (2) KXN 21)(Sysmex