بسم الله الرحمن الرحيم

Sudan University of Science and Technology
Collage of Graduate Studies

Measurement of Complete Blood Counts (CBC) among Sudanese Pregnant Ladies in Elnihood City

قياس تعداد الدم الكامل وسط السيدات السودانيات الحوامل بمدينة النهود

A dissertation submitted in partial fulfillment forthe requirement of MSc Degree in Hematology and Immunohematology

By

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الآية

بسم الله الرحمن الرحيم

(وَقَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ)

صدق الله العظيم

سورة النمل الآية 19

Dedication

To my parents
myhusband
my brothers&sisters
and to everyone how help me in my life.

KHansaa

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First of all thanks Allah for given me the power and willing tocomplete this study.To the spirit of thedeceasedDr. KhaldaM.Hamza whose encourage, guidance and support me from the initial.

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Abstract

This was a case control study, conducted in West Kordofan State at Elnihood City during the period from April to July 2016, which aimed to determine CBC of two hundred and nine (209) healthy Sudanese pregnant women at all trimesters (case) and one hundred and one (101) non pregnant women (control).Subjectswere verbally consented and approved to participate.

Two and half ml of venous blood was collected in EDTA anticoagulated container. Automated hematological analyzer (Urit3010) was used; the results were analyzed by independent T test and one-way ANOVA test using SPSS computer program (version 16).

Age group (20 -35) years was most frequent in both pregnant and non pregnant women(44%,51.5%) respectively, while age group(>35) years was leastfrequent in both study subjects (27%,16.8%) respectively. Grand multigravidae (46.4%) was the highest frequent variable, followed by multigravidae (33%) and primgravidaewas lowest one (20%).

Regard to trimesters third trimester represented most abundant (52%),compared with second trimester (27%)and first one (20%).

One third of pregnant women had history of abortion and most of them received iron supplement (56%).

The studied showed significantstatistical difference of WBCs, RBCs, Hb, HCT, Platelets, Granulocytes and Lymphocytes levels between study group (*P=.000*)for all exceptplatelets(*p=.006*).

There was no significant statistical differencebetween age, abortion and gravidity and WBCs, RBCs, Hb, HCT and platelets.

There was observational differenceWBCs levelsbetween the trimesters. RBCs, Hb, HCT and platelet showed statistical significant correlation in first trimester compared to others, observational correlation of these parameters in pregnant women at third trimester compared to those at second.

مستخلص البحث

تعتبر هذه الدراسة دراسة حالة مقارنة اجريت في مدينة النهود بولاية غرب كردفان في الفترة من ابريل الي يوليو من العام 2016. هدفت هذه الدراسة لقياس صورة الدم الكاملة لدي مائتان وتسعة (209) من النساء الحوامل الاصحاء وفق نظام الاختيار المحدد وعوملن كعينات اختبارية , وتم اختيار مائة و واحد امرة من غير الحوامل الاصحاء وفق نظام الاختيار المحدد وعوملن كعينات ضابطة بعد اخذ اقرار موافقتهن شفاهة.

تم اخذ اثنان ونصف مل عينة دم وريدية من كل متبرعة و وضعت في مانع تجلط (EDTA) وتم اختبارها بواسطة جهاز تحليل الدم الالي ومن ثم تم تحليل النتائج باستخدام برنامج الحزم الاحصائية للعلوم الاجتماعية المحوسب .

الفئة عمرية (20-35) سنة كانت تمثل اغلبية المشاركات من النساء الحوامل وغير الحوامل (44%و52%) علي التوالي,بينما كانت الفئة العمرية(اقل من35) سنة يمثلن اقل عدد من المشاركات من النساء الحوامل وغير الحوامل (27%و11%) علي التوالي.النساء اللاتي سبق لهن الحمل اكثر من ثلاثة مرات كن هن الاكثر شيوعا(46%) ,يليهن اللائي قد سبق لهن الحملن مرتين الي ثلاثة مرات (33%) ثم اللوائي سبق لهن الحمل مرة واحدة(20%).الحوامل في الفترة الثالثة مثلن الغالبية (52%) بالمقارنة مع الفترات الاخري،الفترة الاولي(20%) ،الفترة الثاني(27%) . ثلث الحوامل سبق ان تعرضن لاجهاض (33%) والاغلبية تناولن حبوب الحديد (56%).

دراسة العلاقات الاحصائية ذات الدلالة المعنوية لكريات الدم البيضاءوكريات الدم الحمراء وخضاب الدم والدم المكدس وعدد صفائح الدم والخلايا المحببة والخلايا اللمفاوية بين مجموعات الدراسة توصلت الي انه يوجد اختلاف احصائي ذو دلالة معنوية (p=.000)في الكل ماعدا في الصفائح الدموية (p=.006)

يوجد اختلاف احصائيفي النتائج عندالنساءالحوامل بناءا علياعمارهن وعددمرات الحمل وايضااللاتيتعرضنلإسقاطمقارنةمع اللاتيلميتعرضن لإسقاطفي كريات الدم البيضاء،كريات الدم الحمراء،خضاب الدم، الدم المكدس، عدد صفائح الدم بينما يوجد اختلاف احصائي ذو دلالة وصفية بين كريات الدم البيضاء وفترات الحمل.

دراسة عدد كريات الدم الحمراء، الدم المكدس، تركيز خضاب الدم في الخلية الواحدة وعدد صفائح الدم للنساء الحوامل في الفترة الاولي اظهرت انه يوجد اختلاف احصائيذو دلالة معنوية بالمقارنة مع

الحوامل في الفترات الاخري،بينما هنالك علاقة ذات دلالة وصفية لهذة المتوسطات للنساء الحوامل في الفترة الثانية بالمقارنة مع الحوامل في الفترة الثالثة.

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Abbreviations

B12Cobalamin

CBC Complete blood count

CO_2 Carbon dioxide

DNA Deoxyribonuclicacid

EDTAEthylene-diamine-tetra acetic acid

ESR Erythrocyte sedimentation rate

GITGastro intestinal tract

HbHaemoglobin

Hb F Fatal haemoglobin

HbA_2 Adult Haemoglobin

HCTHaematocrit

IDA Iron deficiency anaemia

MCV Mean cell volume

MCHMean cellhaemoglobin

MCHCMean cellhaemoglobin concentration

O_2 Oxygen

RBC Red blood cell

RDW Red cell distribution width

RNARibonucleic acid

TCIITrans cobalamine two

WBC White blood cell

Chapter One

1.1Introduction:

1.1Pregnancy:

Pregnancy is a state characterized by many physiological and hematological changes, which may appear to be pathological in the non-pregnant state. The review by Chandra highlights most of these changes along with the scientific basis for the same, as per the current knowledge, with a special reference to the red blood and white blood cells, platelets and hemostatic profile(Chandra*etal*.,2012).

1.1.2 Hematological complication:

There are both subtle and substantial changes in hematological parameters during pregnancy and the puerperium, orchestrated by changes in the hormonal milieu(Pavord and Hunt, 2010).

In normal pregnancy, there is an increase in erythropoietic activity. However, at the same time, an increase in plasma volume occurs, this results in a progressive decrease in haemoglobin(Hb), haematocrit(Hct) and red blood cell(RBC). Level returns to normal about a week after delivery. There is a slight increase in mean cell volume (MCV) during the 2nd trimester. Serum ferritin decreases in early pregnancy and usually remains low throughout pregnancy, even when supplementary iron is given (Dacieand Lewis, 2011).

The overall effect of these changes in most women is a slight drop in Hb concentration, which is most pronounced at the end of the second trimester and slowly improves approaching term **(**Kaushansky*et al*., 2016).

Anemia is the most common hematological problem in pregnancy, followed by thrombocytopenia. The Leukocytosis is almost always associated with pregnancy (Dhariwal*et al*., 2016).

In previous study done by Abdalla,determination of complete blood cell count in sudanese Pregnant women in the second trimester in Khartoum Locality his results indicated that significant decrease in Hb and total white blood cells(TWBCs) compared with significant increase in MCV mean of neutrophils of pregnant women compared with non-pregnant women(Abdalla,2012).

1.2 Literature Review:

1.2.1 Hematopoiesis:

Hematopoiesis is the process of blood cell production, differentiation, and development. The hematopoietic system consists of the bone marrow, liver, spleen, lymph nodes, and thymus (Turgeon, 2012).

Blood is a life-sustaining fluid which circulates through the heart and blood vessels. It carries oxygen and nutrients to the tissues and waste products to the lungs, liver and kidneys, where they can be removed from the body(Bain, 2004).

Blood is composed of a pale yellow fluid called plasma in which are suspended red cells (erythrocytes), white cells (leukocytes), and platelets (thrombocytes). Plasma forms about 55% of blood volume and contains water (95%) and many solutes, including proteins, mineral ions, organic molecules, hormones, enzymes, products of digestion, and waste products for excretion (Cheesbrough, 2006).

Table 1.1 Hematological values for normal adults (predominantly from Europe and North America) expressed as a mean _ 2SD (95% range)(Dacie, and Lewis, 2011)

Haematological values	**normal range**
Red blood cell count	Men 5.0 ± 0.5 $\times 10^{12}$/l Women 4.3 ± 0.5$\times 10^{12}$/l
Haemoglobin concentration	Men 150 ± 20 g/l Women 135 ± 15 g/l
Packed c ell volume (PCV) or Haematocrit (Hct)	Men 0.45 ± 0.05 (l/l) Women 0.41 ± 0.05 (l/l)
Mean cell volume (MCV)	Men and women 92 ± 9 fl
Mean cell haemoglobin (MCH)	Men and women 29.5 ± 2.5 pg
Mean cell haemoglobin concentration (MCHC)	Men and women 330±15 g/l
Red cell distribution width (RDW)	
As coefficient of variation(CV)	12.8±1.2%
As standard deviation (SD)	42.5 ±3.5 fl
White blood cell count	4.0–10.0 $\times 10^{9}$/l
Differential white cell count Neutrophils	 2.0–7.0 × 10^{9}/l (40–80%)
Lymphocytes	1.0–3.0 $\times 10^{9}$/l (20–40%)
Monocytes	0.2–1.0 × 10^{9}/l (2–10%)
Eosinophil’s	0.02–0.5$\times 10^{9}$/l (1–6%)
Basophil’s	0.02–0.1$\times 10^{9}$/l (<1–2%)
Platelet count	130-280 $\times 10^{9}$/l

1.2.1.1Red Blood Cells:

Erythropoiesisis the entire process by which red blood cells (RBCs) are produced in the bone marrow (Greer *et al*., 2003).

RBCs are a nucleate, biconcave, discoid cells filled with a reddish protein, hemoglobin (HGB), which transports oxygen and carbon dioxide. It appear pink to red and measure 6 to 8 mm in diameter with a zone of pallor that occupies one third of their center, reflecting their biconcavity (Keohane*et al*., 2016).

1.2.1.1.1RBC in pregnancy:

During pregnancy, the total blood volume increases by about 1.5l, mainly to supply needs of the new vascular bed. Almost 1 liter of blood is contained within the uterus and maternal blood spaces of the placenta. Expansion of plasma volume by 25%–80% is one of the most marked changes, reaching its maximum by mid pregnancy. Red cell mass also increases by 10%– 20% (Pavord and Hunt, 2010).

Red cell volume (or red cell mass) in pregnancyraises from 1400 to 1640mL at term (increase18%), with iron and folatesupplements; an increase of 30% has been reported.The discrepancy between the rate of increase of plasma volume and that of red cell mass results in a relative haemodilution or ‘physiological anaemia’ with the haemoglobin (Hb) concentration, haematocrit, and red cell counts all decrease (particularly in the second trimester).Mean corpuscular Hb concentration remains constant (Collins *et al*., 2013).

1.2.1.1.2Haemoglobin:

Hemoglobin is the life-giving substance of every red cell, the oxygen-carrying component of the red cell (Ciesla, 2007).

Three complex metabolic pathways are required for synthesis of hemoglobin, corresponding to the three structural components of hemoglobin: protein (globin), protoporphyrin, and iron (Greer *et al*., 2003).

The main function of RBCs is to carry oxygen (O_2) to the tissues and to return carbon dioxide (CO_2) from the tissues to the lungs. In order to achieve this gaseous exchange they contain the specialized protein haemoglobin. Each molecule of normal adult haemoglobin A (Hb A) (the dominant haemoglobin in blood after the age of 3–6 months) consists of four polypeptide chains, α2β2, each with its own haem group. Normal adult blood also contains small quantities of two other haemoglobins: Hb F and Hb A2. These also contain α chains, but with γ and δ chains, respectively, instead of β (Hoffbrand and Moss 2016).

Different hemoglobin's are synthesized in the embryo, fetus and adult, each adapted to their particular oxygen requirements.they all have a tetrameric structure made up of two different pairs of globin chains, each attached to one haem molecule (Hoffbrand*et al*., 2005).

1.2.1.1.2.1Haemoglobin in pregnancy:

In normal pregnancy, the physiological change in haemoglobin concentration during pregnancy is well known phenomena. It is also one of the physiological conditions capable ofcausing remarkable and dramatic changes in haematologicalvariables(Ichipi-Ifukor*et al*., 2013).

1.2.1.1.3Packed Cell Volume or Haematocrit:

Hematocrit is the ratio of the volume of packed RBCs to the volume of whole blood;it used to compute the RBC indices (Keohane*et al.*,2016).

1.2.1.1.4 Red Cell Indices:

1.2.1.1.4.1Mean Cell Volume:

In most automated systems, MCV is measured directly, but in semi-automated counters MCV is calculated by

$$MCV = \frac{PCV\ (l\backslash l)\ X1000}{RBCs\ (cell\backslash l)\ x\ 10^{-12}}$$

(Bain ,2004).

1.2.1.1.4.2 Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration:

The MCH gives the amount of haemoglobin in picograms (pg) in an average red cell.

$$MCH = \frac{Hbin\ g/L}{RBCs\ x\ 10^{-12}\ /L}$$

The MCHC gives the concentration of haemoglobin in g/l in 1 litre of packed red cells.

$$MCHC = \frac{Hb\ g/L}{Hct(L\backslash L)}$$

(Cheesbrough, 2006).

1.2.1.1.4.3Red cell distribution width RDW:

The red cell distribution width (RDW) is a mathematical description

of the variation in RBC sizes; a high RDW indicates greater variation in RBC size normal range of RDW= (CV) 12.8 _ 1.2% (SD) 42.5 _ 3.5 fl(Kern, 2002).

1.2.1.2White Blood Cells:

The white blood cells (leucocytes) may be divided into two broad groups: the phagocytes and the lymphocytes. Phagocytes comprise the cells of the innate immune system, which can act very quickly after an infection, whereas lymphocytes mediate the adaptive immune response, which can develop immunological memory, for example after vaccination. Phagocytes can themselves be subdivided into granulocytes (which include neutrophils, eosinophil's and basophil's) and monocytes (Hoffbrand and Moss, 2016).

White blood cells have fundamental roles in defence against invading micro-organisms and the recognition and destruction of neoplastic cells as well as their role in acute inflammatory reactions. Furthermore, through their phagocytic function, white blood cells are influential in clearing senescent and apoptotic cells, hence allowing tissue repair and remodeling(Hoffbrand*et al.*, 2005).

1.2.1.2.1Neutrophils:

Neutrophils have a nucleus is divided into two to five segments or lobes,these lobes are separated by a thin strand or filament of nuclear material. the nuclear chromatin is heterogeneous with some clumping. The cytoplasm of neutrophils is very pale blue and is packed with fine lilac-

staining granules. The granules are referred to as neutrophilic because they owe their colour to uptake of both acidic and basic components of the stain. Neutrophils are produced in the bone marrow; they spend 6–10 hours in the blood stream before moving from capillaries into tissues (Keohane*et al.*, 2016).

1.2.1.2.2 Lymphocytes:

Lymphocytes are the second most common type of leukocytes in adults

(~20–40% of WBC). The lymphocyte number is higher in children and also increases with viral infections (kern, 2002).

1.2.1.2.3WBC in pregnancy:

Pregnancy is associated with alterations in many haematological parameters, one of which is an increase in white blood cell count (leucocytosis), it has been attributed to physiologic stress and increased inflammatory response associated with pregnancy,thisleucocytosis is mainly due to neutrophiliaand immature forms like metamyelocytes and myelocytes(neutrophil left shift) may be present in the peripheral blood film(Pughikumo*et al.*,2015).

1.2.1.3 Platelets:

Platelets are anucleate cells produced in the bone marrow by budding off the cytoplasm of a megakaryocyte. Once released, they circulate for approximately 7 days.It has two main functions in hemostasis:

1. Presentation of a phospholipid surface that is essential for coagulation reactions
2. Formation of a platelet plug at the site of vascular injury(Tefferi, 2001).

1.2.1.3.1Platelet in pregnancy:

Pregnancy is associated with endothelial stress, and increased platelet aggregation in the uteroplacental circulation resulting in a progressive fall in platelet count with increasing gestational age. Also, the increase in plasma volume associated with pregnancy results in a dilutionalthromocytopaenia(Mohamed and Ibrahim, 2016).

1.2.2Pregnancy:

Human gestation(pregnancy) lasts an average of 266 days (38weeks) from conception (fertilization) to parturition (childbirth) (Saladin, 2008).

Clinically, the course of a pregnancy is divided into 3-month intervals called trimesters:

1. The first trimester (first 12 weeks) which is the most precarious stage of development; more than half of all embryos die in the first trimester. Stress, drugs, and nutritional deficiencies are most threatening to the conceptus during this time.
2. The second trimester (weeks 13 through 24) is a period in which the organs complete most of their development.
3. In the third trimester (week 25 to birth), the fetus grows rapidly and the organs achieve enough cellular differentiation to support life outside the womb (Saladin, 2008).

1.2.2.1Heamatological changes during pregnancy:

Hematological profile is considered to be one of the factors affecting pregnancy and its outcome. During pregnancy, changes occur and can be

observed in hematological indices such as red blood cell (RBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Some of these are decreased – for example, RBC and PLT counts – partly as a result of the physiological hemodilution that occurs in pregnancy, while others are increased, such as the WBC count(Akinbami*et al*., 2013).

1.2.2.2Physiological adaptation in pregnancy

• Plasma volume expansion (50%) greater than red cell mass i (25%).

• This leads to physiological dilution with d Hb and HCT.

• A naemia is diagnosed if Hb<10.5 g/dL in pregnancy.

• There should be no change in mean corpuscular volume (MCV) or mean corpuscular haemoglobin concentration (MCHC) in normal pregnancies.

Normally pregnancy has:

• 2 –3-fold increase in iron requirements

• 10–20-fold increase in folate requirements in pregnancy (Collins *et al*.,2013).

1.2.2.3Anemia in pregnancy:

1.2.2.3.1Anaemia:

An anemia is normally defined as a decrease of hemoglobin below13.5 g/dL in males and 11.5 g/dL in females. Between the age of 6 month andpuberty, the normal hemoglobin concentration is somewhat lower (between 11 and 15 g/dL) (Munker*et al*., 2007).

Hb changes may reflect altered plasma volume, not a change in RBC mass, in pregnancy, for example, the plasma volume increases (Greer *et al*., 2003).

Physiologic anaemia is the term often used to describe the fall in haemoglobin concentration that occurs during normal pregnancy results from plasma volume increases above normal by the end of gestation although the red cell masses itself increase by some and still leads to a fall in haemoglobin concentration with a feature of normocytic and normochromic type of anaemia(Das *et al*., 2013).

Anemia during pregnancy is a large health problem in Sudan, where pregnant women in different regions of Sudan are more susceptible to anemia, irrespective of their age or parity (Abdurrahman *et al*., 2012).

1.2.2.3.2 Clinical signs and symptoms of anemia:

The clinical signs and symptoms of anemia can result from diminished delivery of oxygen to the tissues, the lowered hemoglobin concentration (Turgeon, 2012).

Symptoms of decreased oxygen delivery to the central nervous system include light-headedness, somnolence, headache, and lack of attention. Fatigue is a universal complaint but lacks specificity. a gradual onset of anemia, regardless of severity, may be relatively asymptomatic because of physiologic adjustment by the body, Mucocutaneous pallor signifies decreased hemoglobin and is appreciated best in the conjunctiva, nail beds, and roof ofthe mouth also special sings may present in different type of anemia (koilonychia in iron deficiency)(Tefferi, 2001).

1.2.2.3.3 Classification of aneamia:

Generally, anemias are classified either morphologically or according to pathophysiological cause. The pathophysiological approach refers to the cause of anemias—whether the anemia is caused by excessive destruction or diminished production of red cells, clinicians are familiar with the morphological classification of anemias that relies on the red blood cell indices (Table1.2)(Ciesla, 2007).

Table1.2 morphologicalclassification of anaemia (Hoffbrand and Moss, 2016)

Microcytic,hypochromic	**Normocytic,normochromic**	**Macrocytic**
MCV <80 fL	MCV 80–95 fL	MCV >95 fL
MCH <27 pg	MCH ≥27 pg	Megaloblastic vitamin B12 or folate deficiency Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anaemia, etc.
Iron deficiency	Many haemolyticanaemias	
Thalassaemia Anaemia of chronic disease (some cases) Lead poisoning Sideroblasticanaemia (some cases) 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.)	

1.2.2.3.4 The most common type of anemia in pregnancy:

1-Iron deficiency anemia.

2-Megalopastic anemia.

1.2.2.3.4.1 Iron-deficiencyanemia:

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributable to iron deficiency and accounts for ~841,000 deaths annually worldwide.

Africa and parts of Asia bear 71% of the global mortality burden; North America represents only 1.4% of the total morbidity and mortality associated with iron deficiency(Longo, 2010).

Iron deficiency anemia (IDA) is a major health problem during pregnancy and it has adverse effects on the mother and the newborn (Abdelrahman*et al*., 2012).

The most common cause of anaemia in pregnancy (90% of cases) often asymptomatic and detected on screening (Kern, 2002).

1.2.2.3.4.1.1Iron requirement:

Iron is lost through cells lining the GITtract (gastro intestinal tract) and through the superficial squamous cells of the skin as they are shed; a small amount of iron is alsolost in sweat. This obligatory iron lossaverages approximately 1 mg per day in adult men and postmenopausal women and approximately 2 mg per day in menstruating women. Times of increased iron need include the first 18 months of life (particularly in premature infants), the adolescent growth spurtand pregnancy.Woman loses approximately 750 mg during the average term pregnancy; approximately

225 mg of this is taken by the fetus,mostly during the last trimester (Kern, 2002).

1.2.2.3.4.1.2Causes of iron deficiency:

Conditions that increase demand for iron, increase iron loss, or decrease iron intake or absorption can produce iron deficiency (Longo, 2010).

Table 1.3Causes of iron deficiency:(Longo, 2010)

Causes of iron deficiency
Increased demand for iron and/or hematopoiesis
Rapid growth in infancy or adolescence
Pregnancy
Erythropoietin therapy
Increased iron loss
Chronic blood loss
Menses
Acute blood loss
Blood donation
Phlebotomy as treatment for polycythemia vera
Decreased iron intake or absorption
Inadequate diet
Malabsorption from disease (sprue, Crohn’s disease)
Malabsorption from surgery (post-gastrectomy)
Acute or chronic inflammation

1.2.2.3.4.1.3Diagnosis

An early event in iron deficiency is a decrease in serum ferritin(the major

iron storage protein) also decrease in the hemoglobin concentration. At this point, the number of red blood cells can still be normal. If the iron deficiency persists, abnormalities of red cell morphology can be recognized (e.g., poikilocytosis, anisocytosis, hypochromasia),while reticulocytes are generally decreased and the red cell indices (e.g., MCV, MCH, and MCHC) are below normal. The bone marrow morphology shows nonspecific changes(Munker*et al*., 2007).

1.2.2.3.4.2 Megaloblasticanaemia:

Megaloblastic anemia is defined by a highly characteristic set of morphological changes which affect cells of the erythroid, and megakaryocytic lineages in the peripheral blood and bone marrow. These changes include macrocytosis, Howell–Jolly bodies, hyper segmented neutrophils, giant metamyelocytes and giant platelets (Provan and Gribben, 2005).

1.2.2.3.4.2.1Causes of megaloblasticanaemia:

1.Vitamin B12 deficiency :

It can be due to:

1. Decreased in intake:dietary, impaired absorption, malabsorption and competition from parasites.
2. Increased requirements:pregnancy, increased cellular proliferation, hyperthyroidism .

3. Impaired utilization: Red cell enzymopathy, abnormal vitamin B12 binding protein, Nitrous oxide administration, Lack of transport protein (TcII).

2. Folatedeficiency:

It may result from:

1. Decreased in intake: dietary, alcoholism, impaired absorption due to sprue and celiac disease
2. Increased requirements: pregnancy, increasedcellular proliferation, miscellaneous states (Homocystinuria, hyperthyroidism)
3. Impaired utilization: folic acid antagonists (methotrexate, dilantin, trimethoprim,pyrimethamine)(Abdul hamid,2013).

1.2.2.3.4.2.2 Laboratory diagnosis:

CBC shows a pancytopenia (low white count, low red count, and low platelet count).Which in the CBC combined with macrocytosis should raise the index of suspicion toward a megaloblastic process because few other conditions (aplastic anemia, hypersplenism) show this pattern. Red cell inclusions such as basophilic stippling and Howell-Jolly bodies may be observed.There is a low reticulocyte count (less than 1%) and the RDW is increased, owing to schistocytes, targets, and teardrop cells. The blood smear in megaloblastic anemia is extremely relevant in the diagnosis and shows macrocytes, macro-ovalocytes, hypersegmentedmultilobed neutrophils, and little polychromasia with respect to the anemia .The presence of hyper-aged to increase their folic acid intake because decreased folatemay lead to neural tube defects (Ciesla, 2007).

1.3 Previous studies:

Many researches were conducted in the world to evaluate hematological profile in normal pregnancy.

Hematological profile of pregnant women studied byOsonugaand his colleagues (2011), in Southwest of Nigeria, showed that statistically significant lower values of PCV, monocyte and lymphocyte while WBC, eosinophil and ESR were not significantly changed. There was no significant difference in all hematological parameters among the three trimesters.

Study done in Sudan byElhussein(2015**),** Measured Complete Blood Cell Count of Sudanese pregnant women at Third Trimester Khartoum Locality, concluded that: there was significantdecreased in means of HCT, TRBCs, RDWSD, lymphocytes percentage and absolute count and platelets count in pregnant women when compared with control**,** significant increase in means of MCHC, TWBCs and neutrophils percentage and absolute of pregnant women more than control.

Other study done by Idris(2016)determined complete blood Cell Count of Sudanese pregnant women at second Trimester Khartoum North Hospitals, results showedsignificantdecreasein mean of HCT, TRBCs, Hb, and MCHC and lymphocytes percentagein pregnant women when compared withnon-pregnant women.TWBCs, PDW, MPV and neutrophils percentage significantly increase. Insignificant decrease in means of MCV, MXD% and Platelets in pregnantwomen when compared with non-pregnant women.Insignificant increase in means of MCH and RDW/sd.

Abdalgader(2015), in her study measuredcomplete blood Count among Sudanese pregnant women attended in Military Hospital Omdurman,

showed that there was significant difference between trimester and RBC,HCT, WBC, lymphocyte,neutrophil andplatelet. .

1.4 Rationale

Pregnancy is a state characterized by many physiological and hematological changes, which may appear to be pathological in the non-pregnant state (Chandra*et al*.,2012).

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 (Abdurrahman *et al*., 2012)

Maternal mortality is unacceptably high. About 830 women die from pregnancy- or childbirth-related complications around the world every day, occur mainly in developing country.The high number of maternal deaths in some areas of the world reflects inequities in access to health services, and highlights the gap between rich and poor. Women die as a result of complications during and following pregnancy and childbirth. Most of these complications develop during pregnancy and most are preventable or treatable. (WHO, 2016)

This research is base line data to formulate clinical files about hematological status of Sudanese pregnant women.

1.5 Objectives of the study:

1.5.1 General Objective:

To measurecomplete blood count of Sudanese pregnant women in Elnihood city.

1.5.2 Specific Objectives:

1-To measurered blood cells,haemoglobin level ,haematocrit, red blood cell indices, white blood cells,lymphocyte %, lymphocyteabsolute, neutrophil %, neutrophil absoluteand platelets of pregnant women and nonpregnant women.

2-To correlate complete blood count with possible factors affecting study subjectssuch as: age group, abortion, gravidity.

3-To compare WBCs, RBCs, Hb, HCT, PLT in pregnant women in different trimesters.

Chapter Two

Materials and Methods

2.1 Study design:

This was a case control studyconducted in period from April to July (2016).

2.2Study area:

Study conducted in Elnihood city in West KordofanState, Sudan.

2.3 Study population:

Study carried out inSudanese pregnant women as study groupand non pregnant women as control in same age group.

2.4Inclusion criteria:

Healthy pregnant women in the all trimesters, and age group ranged from15-45years,where included after verbal inform consent.

2.5. Exclusion criteria:

Presence of any diagnostic diseases such as anemia,typhoid, malaria or recent previous bloodtransfusion, abortion in the last 4 months were excluded.

2.6. 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.

2.7. Sample SizeandSampling technique:

Non Probability Voluntary sampling were taken , total sample size 310 Sudanese pregnant women 209 as study group at different trimesters and 101non pregnant as control in same age group.

2.9. CBC determination:

Full automated cell counter "Urit3010". (See appendix)

2.10 Blood test and procedure:

2.10.1 Specimen collection:

Two and half mlvenous blood was collected in EDTA containerfrom all participants andcomplete blood count (CBC) was done. well and genital mix of sample before examine and the sample analyzed within 2hrs at roomtemperature.Clotted sample wasavoided.

2.10 Method:

2.10.1 CBC measurement:

Procedure of complete blood count (CBC):

Automated Blood Cell Analysis:

Hematology analyzers available from different manufacturers, most rely on only two basic principles of operation: electronic impedance (resistance) (Electronic impedance, or low-voltage direct current (DC) resistance, is the most common methodology used)and optical scatter (Optical scatter,using both laser and non-laser light, is frequently employed in today's hematology instrumentation) (keohane*etal*., 2016).

2.10. Quality Control (QC):

One sample of blood was investigated for CBC in many laboratories in Elobaid and Elnihood city.

2.11. Ethical consideration:

Verbalinform consent from selected subjectswas taken after being informed with all detailed objective of the study, the result was given to all subjects, and the anaemic women was given folic acid or fefol.

2.12. Statistical analysis:

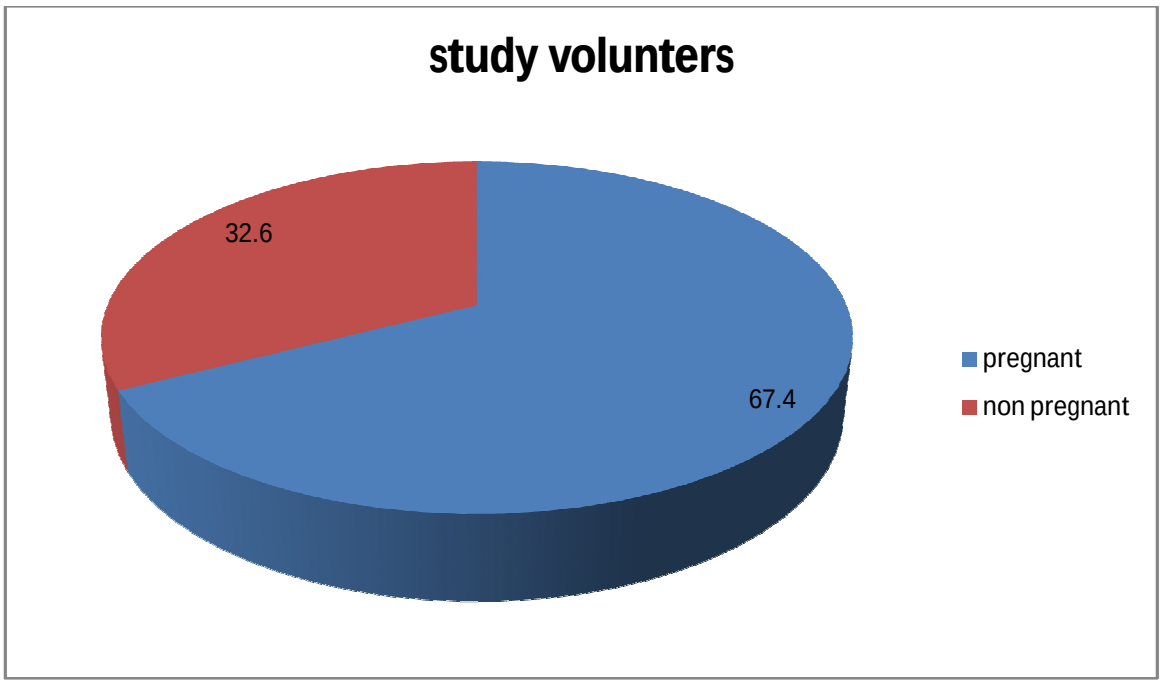
Data entered checked and analyzed using Statistical Package of Social Sciences (SPSS) software program(version16). Data analyzed by (one way ANOVA and Independent T-test), used *P-value* significant level was set at $\leq$ 0.05.

Chapter Three

Result

Total of 310 volunteers whom approved to participate in this study attended to Elnihood city classified to (209/ 310, 67.4 %) pregnant women and (101/310, 32.6%) non pregnant women (Table 3.1)

Figure 3.1: Distributionof study volunteers



Age group (20 -35) years was most frequent in both pregnant and non pregnant women (44 %) and (51.5%), while age group (>35)years showed lower frequency in both case and control (27.3%) and (16.8%) respectively(Figure 3.2,3.3).

Figure3.2&3.3: Frequency of age group in both pregnant and non-pregnant women

Figure 3.2age group in pregnant women

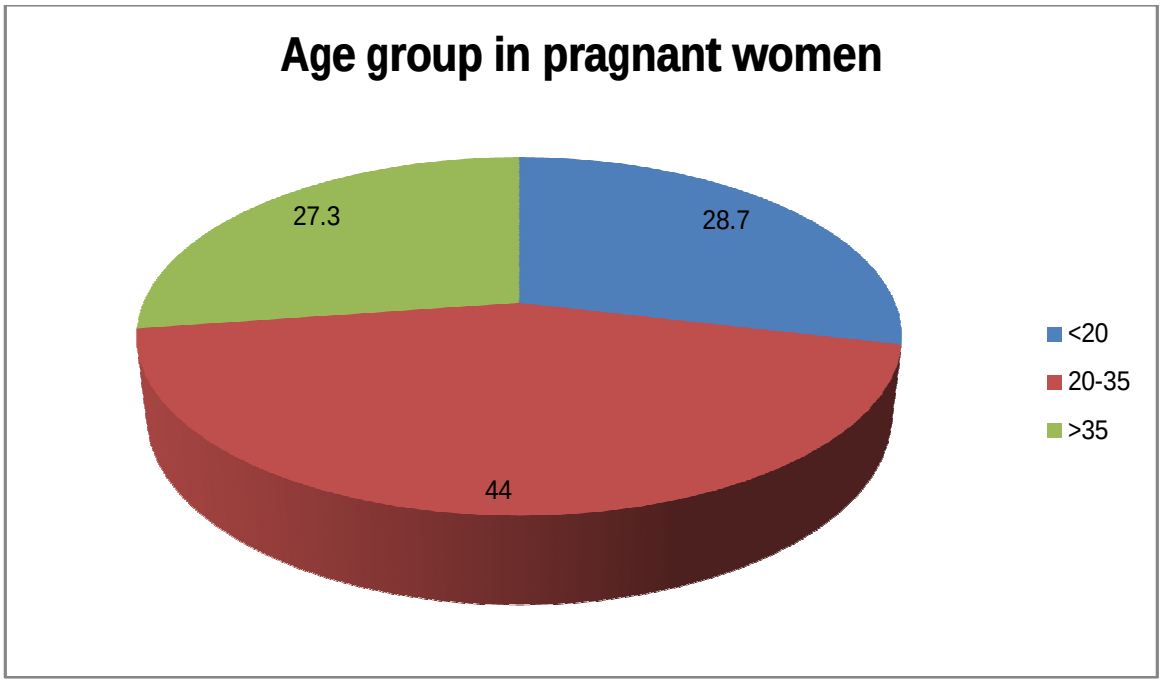
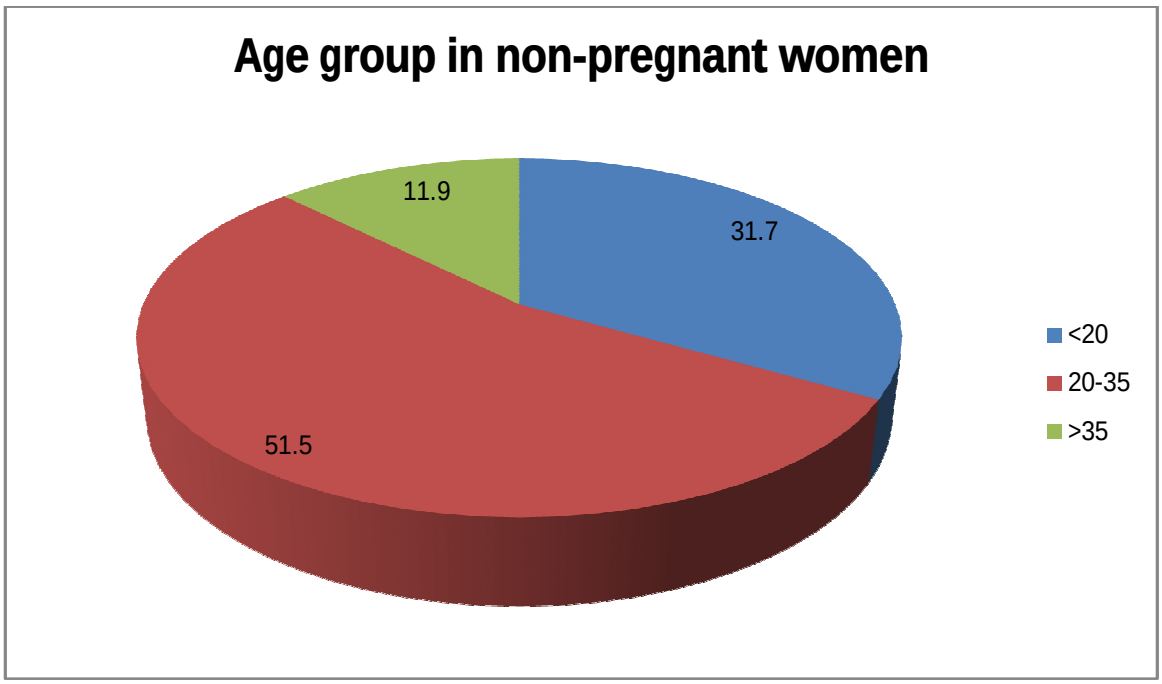


Figure 3.3age group non-pregnant women



Distribution of Pregnant women according to gravidity showed that grand multigravidae (97/46.4%) was highest frequent variable, followed by Multigravidae(70/33.5%)while primgravidaewas lowest one (42/20.1%). Regard to trimesters third trimester represented most abundant (110/52.6). Compared with other trimestersfirsttrimester (42/20.1) and Second (57/27.3).

Only (70/33.5%) of pregnant women had history of abortion (Figure3.4,3.5,3.6)

Figure 3.4, 3.5, and 3.6:Frequancy of possible risk factors in pregnant woman

Figure 3.4Distribution of Pregnant women according to gravidity

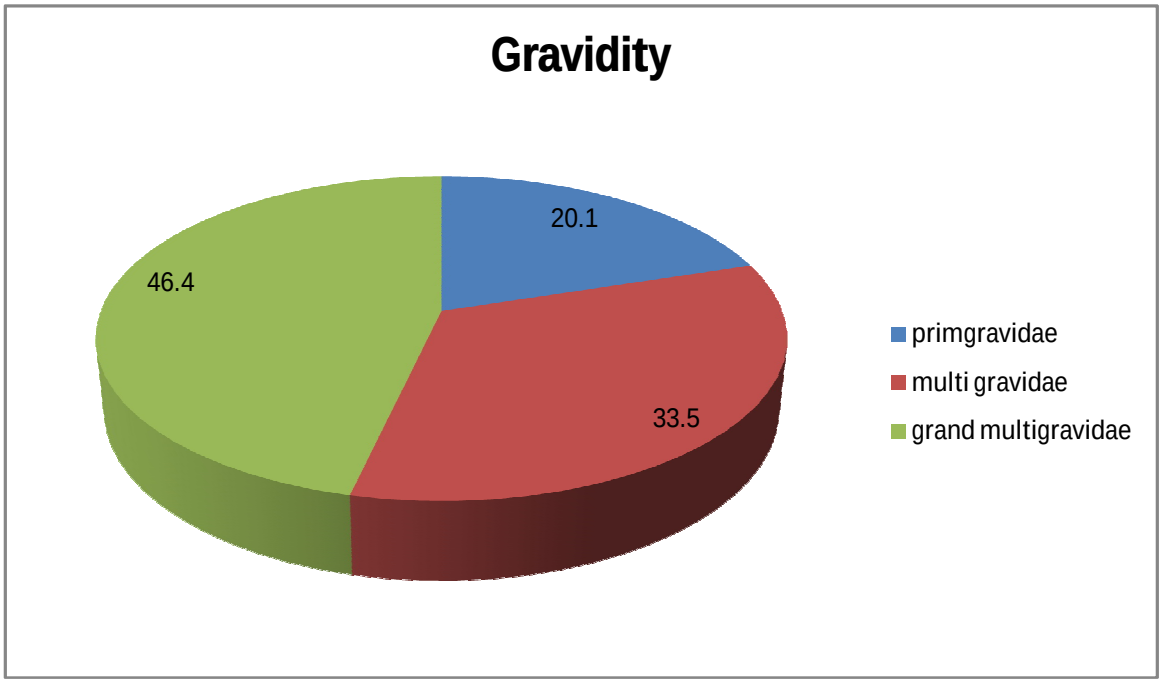


Figure 3.5Distribution of Pregnant women according to trimesters

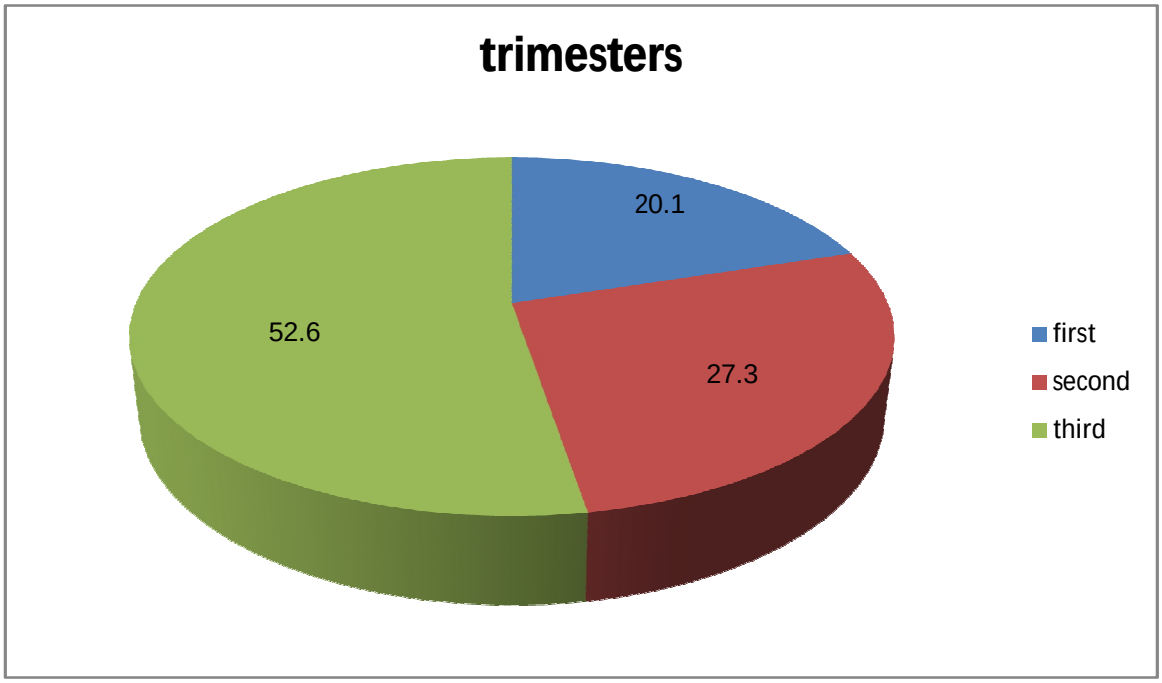
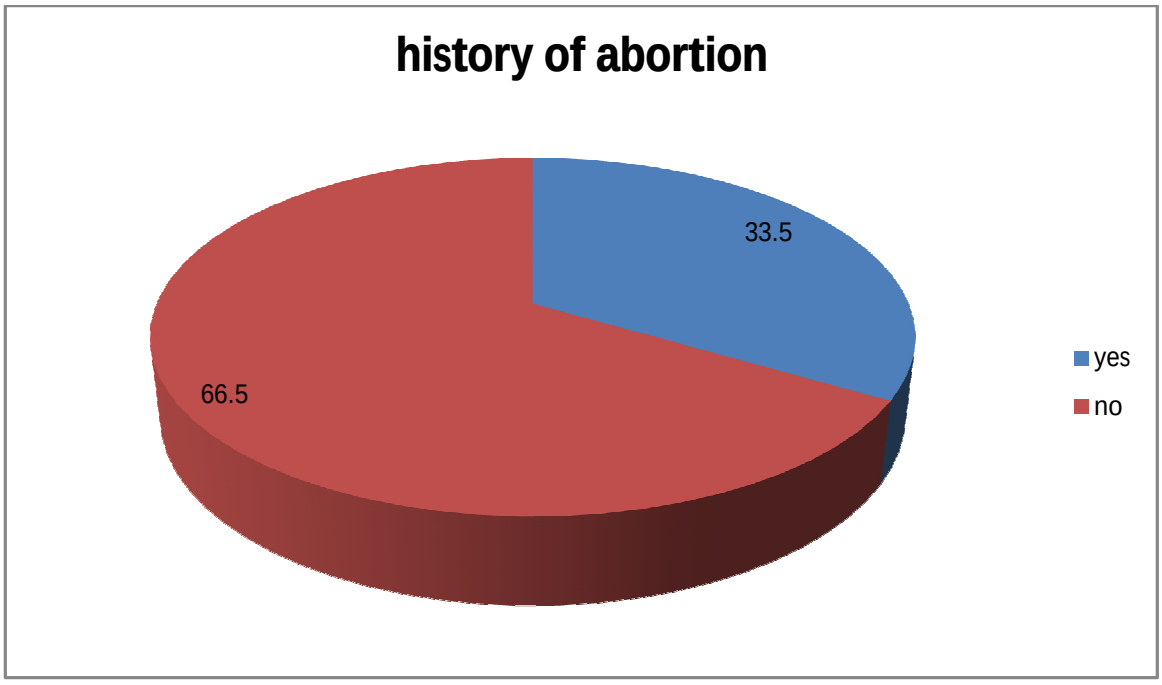
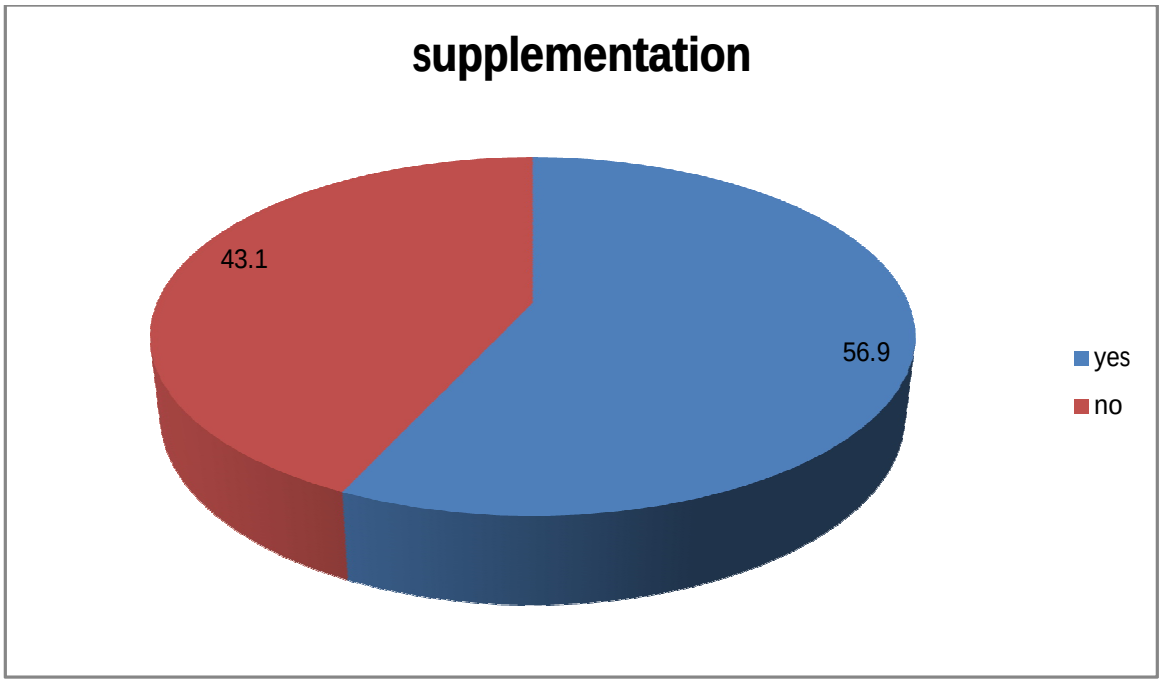


Figure 3.6 Distribution of Pregnant women according tohistory of abortion



Majority of pregnant received iron supplement (119/56.9%)(Figure 3.7)

Figure 3.7Supplementation intake



Complete Blood Count of pregnant women and non- pregnant women:

Mean of RBCs, Hb and HCT and RBC indices except MCVand MCHC showed statistical correlation between study groupstable 3.1.

The mean showed statistical difference in all WBCs type and platelettable 3.2.

There was no difference between age of pregnant women and WBCs, RBCs, Hb, HCT and platelet count, for example *p-value* of WBCs of age group <20&20-35 (*P.V =.811*), RBCs of age group<20 &>35(*P.V=.584*) and platelet of age group20-35&>35 (*P.V=.455*)table 3.3.

No difference was found betweengravidity and WBCs, RBCs, Hb, HCT and platelet count, for example.WBCs in primgravidae compared to multigravidae (*P.V =.644*), RBCs inmultigravidaecompeared grand multigravidae (*P.V=.669*) table 3.4.

There was no difference between history of abortion and WBCs, RBCs, Hb, HCT and platelet count, for example.WBCs(*P.V =.694*), RBCs (*P.V=.954*) table 3.5.

No difference was found between WBCs and trimesters, while there was correlation between RBCs, Hb, HCT and platelet in pregnant women at first & second first and third trimesters and between pregnant women at first & third trimesters table 3.6.

Complete Blood Count of pregnant women and non- pregnant women:

Table3.1: Mean of CBC in pregnant women compared to non-pregnant women

Parameter	**Pregnant Mean± S.D**	**non-pregnant Mean± S.D**	***p-value***
RBC×10^{12}/L	4.04±.36	4.37±.43	*.000*
Hb g/dl	11.33±1.17	11.90±1.30	*.000*
HCT%	30.93±3.55	32.818±4.09	*.000*
MCV fl	76.67±7.46	75.56±7.77	*.228*
MCH pg	28.05±2.47	27.33±2.52	*.018*
MCHC g/l	36.85±2.53	36.33±2.18	*.080*

Table 3.2: Mean of WBCs (granulocyte, lymphocytes) and Platelet count of study volunteers

Parameter	**Pregnant Mean± S.D**	**non-pregnant Mean± S.D**	***p-value***
WBC10^{9}/L	7.05±2.02	5.86±1.94	*.000*
Granulocyte %	66.12±8.32	52.90±10.88	*.000*
Granulocyte absolute	4.74±.53	3.21±.64	*.000*
Lymphocyte%	28.12±8.53	40.388±11.05	*.000*
Lymphocyte absolute	1.90±1.75	2.249±1.69	*.000*
Platelet 10^{9}/L	279.94±63.69	301.92±70.03	*.006*

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

Parameter	**<20** **Mean± S.D**	**20-35** **Mean± S.D**	**>35** **Mean± S.D**
WBCs×10^9/L	6.99±1.96	7.07±2.04	7.08±2.08
RBC×10^{12}/L	4.06±.38	4.04±.36	4.02±.34
Hb g/dl	11.48±1.02	11.20±1.29	11.38±1.10
HCT%	30.8±3.26	30.92±3.88	30.98±3.29
Platelet×10^9/L	279.63±64.17	283.11±66.37	275.02±59.26

Table 3.4: Effect of gravidity on WBCs, RBCs, Hb, HCT and PLTsduring pregnancy

Parameter	**Primgravidae** **Mean± S.D**	**Multigravidae** **Mean± S.D**	**grand multigravidae** **Mean± S.D**
WBCs×10^9/L	7.30±1.84	7.12±2.03	6.90±2.10
RBC×10^{12}/L	4.08±.39	4.04±.35	4.02±.35
Hb g/dl	11.46±1.03	11.49±.97	11.16±1.33
HCT%	30.71±3.21	31.74±3.16	30.44±3.85
Platelet×10^9/L	298.40±59.82	273.43±67.49	276.65±61.60

Table 3.5:Effect of history of abortion on WBCs, RBCs, Hb, HCT and PLTs during pregnancy

Parameter	**With history of abortion Mean± S.D**	**Without history of abortion Mean± S.D**
WBC10^9/L	6.97±2.05	7.09±2.02
RBC×10^{12}/L	4.04±.34	4.04±.37
Hb g/dl	11.27±1.13	11.36±1.19
HCT%	30.67±3.56	31.06±3.54
Platelet 10^9/L	274.79±72.39	282.54±58.93

Table 3.6: Comparison between trimesters and WBCs, RBCs, Hb, HCT and PLTs

Parameters	**1st.T Mean± S.D**	**2ed.T Mean± S.D**	**3rd.T Mean± S.D**	***P.Value***		
				1st.T&2ed .T	***1st.T&3rd .T***	***2ed.T&3rd .T***
WBC×10^9/L	6.58±1.59	7.24±1.80	7.138±2.26	*.106*	*.130*	*.737*
RBC×10^{12}/L	4.31±.30	3.95±.32	3.99±.36	*.000*	*.000*	*.451*
Hb g/dl	12.04±.99	11.17±1.17	11.14±1.15	*.000*	*.000*	*.864*
HCT%	32.30±3.29	30.57±3.95	30.59±3.33	*.016*	*.008*	*.970*
Platelet×10^9/L	306.17±61.10	280.51±57.75	269.64±65.20	*.045*	*.001*	*.287*

1st.T=first trimester, 2ed.T=second trimester, 3rd.T=third trimester.

Chapter four

Discussion, Conclusion andRecommendations

4.1 Discussion

The present study showed that age group(20-35) yearswas the most frequent and while (>35) showed least frequent both pregnant and non pregnant women, bothgrand multigravidaeand third trimester were mostabundant and only about one third of pregnant women had history of miscarriage and most of them received iron supplement, This agreed with (Osman, 2017).Abortion, ageand gravidityhave no correlation on the result this agree with(Awadelkareem, 2015)said that no significant effect of abortion, number of children and age on CBC of pregnant women.

In present study RBC, Hb, HCT,Lymphocyte and platelet showed significant statistical differencein pregnant women compared to control, similar finding obtained by (Mohamed &Ibrahim 2016).This result as the totalblood volume increases by about 1.5 l, mainly to supply the needs of the new vascular bed.(Pavord and Hunt, 2010)

MCH showed significant differencewhileMCV and MCHC showed observationalcorrelation betweencompartive group, this agree with(Dhariwal*et al.*, 2016)and dis agree with(Awadelkareem,2015),This agreement may be due to variation supplementation during pregnancy(Ichipi-Ifukor*et al.*, 2013).

WBCs and Granulocytes showedstatisticalsignificantdifferencein pregnant women compared to non-pregnant subjects;This agreed with (Pughikumo*et al.*, 2015) and disagrees with (Ichipi-Ifukor*et al.*, 2013).

Increase in white blood cell count t has been attributed to physiologic stress and increased inflammatory response associated with pregnancy.This leucocytosis is mainly due to neutrophiliaand immature forms (Canzoneri*et al.*,2009;Crouch *et al.*,1995).

There was observational differencein WBCs levels between the trimesters, this agrees with (Pughikumo *et al.*, 2015)They demonstrated a progressive increase in WBC count with gestational age.and disagree with (Akinbami*et al.*, 2013).

In present study RBCs, Hb, HCT, platelet showed statisticalsignificant difference between first and others, this agree with (Akinbami*et al.*, 2013;Mohamed and Ibrahim 2016) said that this parameters decrease with increasing gestational age , andthere was no significant correlation between second and third trimester this agree with (Tahir,2015),because in late pregnancy, plasma volume increases at a slower rate, inducing a slight rise in hematocrit level.(Shen*et al.*, 2010)

4.2 Conclusions:

1-pregnancy associated with low level of RBCs, Hb, HCT, Lymphocyte and platelet, and increased WBCs and granulocytes levels.

2- RBCs, Hb, HCT and platelet decreased with increasing gestational age.

3- Age, Abortion and gravidity didn’t affect WBCs, RBCs, Hb, HCT and platelet level in study group.

4.3 Recommendations:

1. Providinghealth care and follow-up free or within minimum cost in Sudan, especially in rural areas.

2. Further studies to establish data base for hematological references values for pregnant women.

3. Public health awareness in peripheral areas.

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APPENDEX (1)

Sudan University of Science and Technology

College of Graduate Studies

(Measurement of Complete Blood Counts (CBC) among Sudanese Pregnant Women in Elnihood City)

ID:

Age: …….

Residence: ……

Pregnant : YES ( ) NO( )

Trimester FirstSecond Third

NO of pregnancy: …….

Abortion: yes ( ) date( )No ( )

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

Supplementation intake: yes ( ) Regular ( ) Irregular ( ) No ( )

Why irregular? …….

Visit to clinic yes ( ) No ( )

Nutritional status: Bad ( )Good ( )

Economical status: Bad ( )Good ( )

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

Result:

WBC….....RBC……HGB……..……..

HCT……....MCV……...MCH……..…….

MCHC……..…PLT……LYM%.....

Granulocyte%.....LYM#.....Granulocyte#.....…

APPENDEX (2)

URIT 3010