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**Determination of Hemoglobin and Serum Ferritin in anemic pregnant women and their newborns in Khartoum state**

قياس مستوى خضاب الدم و فريتين المصل في النساء الحوامل المصابات بفقر الدم و اطفالهن حديثي الولادة بولاية الخرطوم

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

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآية:

قال تعالى:

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ.)

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## **Dedication :**

I dedicate my dissertation work to my family, A special feeling of gratitude to my loving parents. To my wife and daughters, To my sisters and brothers, To my relatives and friends, Who have supported me throughout the process. I will always appreciate all they have done.

## **Acknowledgment :**

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## Abstract

Anemia is a global public health problem, it occurs at all stages of life cycle but is more prevalent in pregnant women and young children. The major function of hemoglobin is to carry oxygen from the lung to tissues and carbon dioxide from tissues to the lung.. Ferritin is a ubiquitous intracellular protein that store iron and release it in a controlled fashion.

This is a case control prospective study conducted in Khartoum State during the period between April to August 2015. The aim of this study is to measure hemoglobin and serum ferritin levels in newborns of anemic mother as case and non-anemic mothers as control at the time of delivery and to determine relationship between hemoglobin and serum ferritin of mothers and their newborns at the time of delivery.

Self report structured interview was designed and blood sample were collected from 70 anemic pregnant women aged between 21-40 years before labor (pre-delivery) as case (Hb <11.0 g/dL), another 70 blood sample were collected from non-anemic pregnant women ( $\geq 11.0$  g/dL) age between 21-40 before labor as control group. After delivery blood sample was taken from their newborns.

Hemoglobin was measured using automatic blood counter (Sysmex KX-21N)

Serum ferritin was estimated by using (Cobas 411 e) instrument .

The results showed anemic mothers (cases) hemoglobin mean was  $9.2 \pm 0.72$  g/dl and the mean of ferritin of cases was  $7.8 \text{ ng/ml} \pm 8.6$  and the mean of

hemoglobin for newborns of cases was  $14.53 \text{ g/dl} \pm 1.34$  and the mean value of serum ferritin for newborns of cases was  $72.4 \text{ ng/ml} \pm 27.5$ .

The mean of hemoglobin of non-anemic mothers was  $11.6 \text{ g/dL} \pm 0.63$  and the average of ferritin was  $14.31 \text{ ng/mL} \pm 9.32$ .

The mean of hemoglobin for newborn of control was  $15.43 \text{ g/dl} \pm 1.22$  and the mean value of serum ferritin for newborns of control was  $96.7 \text{ ng/ml} \pm 29.3$ .

The study concluded that there is a significant differences in the mean value of Hb among newborns of anemic mothers and non-anemic mothers (P.value = 0.039), There is also a significant differences in the mean value of serum ferritin among newborns of anemic mothers and non-anemic mothers (P.value = 0.001) and there were a significant positive association between Hb and serum ferritin of anemic (case group) mothers and their newborns .also there is a positive association of Hb and serum ferritin of non-anemic (control group) mothers and their newborns. The study showed that the maternal anemic pregnant women affect Hb and serum ferritin of their newborns .

The study recommends that there should be a regular investigations for pregnant women for hemoglobin and serum ferritin to ensure the wellbeing of pregnant women which reflects on their newborns.



## المستخلص

فقر الدم من أهم المشكلات الشائعة عالمياً والتي تحدث في جميع مراحل الحياة وهو أكثر انتشاراً بين النساء الحوامل والأطفال. وظيفة خضاب الدم الرئيسية هي نقل الأكسجين من الرئتين إلى أنسجة الجسم المختلفة ونقل ثاني أكسيد الكربون من الأنسجة إلى الرئتين. الفريتين هو بروتين متواجد بصورة دائمة داخل الخلايا ووظيفته تخزين عنصر الحديد وإخراجه بطريقة منتظمة حسب حاجة الجسم لعنصر الحديد.

هذه دراسة إحصائية لحالات و ضوابط، أجريت الدراسة في ولاية الخرطوم في الفترة ما بين إبريل- أغسطس 2015. الهدف من الدراسة هو قياس خضاب الدم وتركيز الفريتين للأطفال حديثي الولادة من أمهات مصابات بفقر الدم وأخريات غير مصابات بفقر الدم (مجموعة الضبط) وتحديد العلاقة بين خضاب الدم وتركيز الفريتين بين الأمهات و مواليدهن الجدد.

جمعت البيانات المطلوبة عن طريق المقابلات والاستبيانات. وتم سحب عينات دم من 70 امرأة حامل مصابة بفقر الدم و 70 امرأة حامل غير مصابة بفقر الدم و تراوحت أعمارهن بين 21-40 سنة. وبعد الولادة تم سحب عينات دم من مواليدهن الجدد وتم قياس خضاب الدم عن طريق جهاز (Sysmex KX-21N) ، و قياس تركيز الفريتين عن طريق جهاز (Cobas 411 e) .

أظهرت النتائج التي تم الحصول عليها أن متوسط خضاب الدم للامهات المصابات بفقر الدم  $9.2 \text{ g/dl} \pm 0.72$  ومتوسط الفريتين  $7.8 \text{ ng/mL} \pm 8.6$  ، و كان متوسط خضاب الدم لمواليدهن  $14.53 \text{ g/dL} \pm 1.34$  و متوسط تركيز الفريتين  $72.4 \text{ ng/mL} \pm 27.5$  ، كما أظهرت النتائج أن متوسط خضاب الدم للأمهات الغير مصابات بفقر الدم (مجموعة الضبط)  $11.6 \text{ g/dL} \pm 0.63$  ومتوسط تركيز الفريتين  $14.31 \text{ ng/mL} \pm 9.32$  ، و كان متوسط خضاب الدم لمواليدهن  $15.43 \text{ g/dL} \pm 1.22$  و متوسط تركيز الفريتين  $96.7 \text{ ng/mL} \pm 29.3$  .

وخلصت الدراسة أن هناك علاقة ذات دلالة إحصائية في معدلات خضاب الدم بين مواليد الأمهات المصابات بفقر الدم و مواليد الأمهات الغير مصابات بفقر الدم (النسبة المعنوية 0.039)، وأن هناك علاقة ذات دلالة إحصائية في معدلات تركيز فريتين الدم بين مواليد الأمهات المصابات بفقر الدم و مواليد الأمهات الغير مصابات بفقر الدم (النسبة المعنوية 0.001)، مع وجود علاقة طردية بين خضاب الدم و تركيز الفريتين للأمهات المصابات بفقر الدم و مواليدهن الجدد . كما أوضحت الدراسة وجود علاقة طردية بين متوسط خضاب الدم وتركيز الفريتين للأمهات الغير مصابات بفقر الدم (مجموعة الضبط) و مواليدهن.

أظهرت الدراسة أن الأمهات المصابات بفقر الدم أثناء الحمل يؤثر على مستوى خضاب الدم و تركيز الفريتين لمواليدهن الجدد.

أوصت الدراسة بأهمية قياس خضاب الدم وتركيز الفريتين للنساء الحوامل باستمرار حفاظاً على صحتهن و التي تنعكس على المواليد.

## List of Abbreviations

CBC	Complete blood count
CO <sub>2</sub>	Carbon dioxide
DMT1	Divalent metal transporter one
dL	Deciliter
EDTA	Ethylene Di-amine Tetra Acetic Acid
Fe	Iron
Fe <sup>+2</sup>	Ferrous iron
Fe <sup>+3</sup>	Ferric iron
g	Gram
G6PD	Glucose 6-phosphate dehydrogenase
Hb	Hemoglobin
HCO <sub>3</sub>	Bicarbonate
HIV	Human immunodeficiency virus
ID	Iron deficiency
IDA	Iron deficiency anemia
Kg	kilogram
L	liter
LCD	Liquid crystal display
MCV	Mean corpuscle volume
MCHC	Mean corpuscle hemoglobin concentration
mL	Milliliter
ng	Nanogram
O <sub>2</sub>	Oxygen
pCO <sub>2</sub>	Partial pressure of carbon dioxide
PCV	Packed cell volume
pO <sub>2</sub>	Partial pressure of oxygen
pH	Hydrogen number
RBCs	Red blood cells

RES	Reticuloendothelial system
Rh	Rhesus
Sf	Serum ferritin
Tfr	Transferrin receptor
WBCs	White blood cells
WHO	World Health Organization

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# **Chapter one**

Introduction and  
Literature Review

# 1. Introduction and Literature Review

## 1.1 Introduction :

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of life cycle but is more prevalent in pregnant women and young children. It occurs when the concentration of hemoglobin falls below what is normal for a person's age, gender and environment, resulting in the oxygen carrying capacity of the blood being reduced. Anemia is often classified as mild degree (Hb 10.0-11.0 g dL), moderate ( Hb 7.0-9.9 g dL), severe (Hb 4.0-7.0 g dL) and very severe Hb <4.0 g dL. ( WHO, Geneva, 1992)

Anemia in pregnancy is a major challenge to obstetric care in developing countries where the prevalence rate varies between 30% to 70%. Since the prevalence of anemia in non-pregnant women in developing countries is also high 40%. which is possible for these non-pregnant women were already anemic at the time of conception. causes of anemia during pregnancy are multi factorial this include nutritional deficiencies iron, folate and vitamin B12, and parasitic disease such as malaria in endemic areas like Sudan and hookworm infestation (VanderJagt *et al.*, 2007). also fluid overload (hypervolemia) cause decrease hemoglobin concentration and apparent anemia. however iron deficiency is believed to be the main underlying cause for anemia in pregnancy due to increase in demand of iron for fetal and maternal tissue growth . ( WHO, Geneva, 1992 ).

Iron deficiency during pregnancy has been associated with multi adverse outcomes for both mother and infant, including increase risk of maternal mortality and prenatal mortality and low birth weight (Scholl, 2005).

Folate deficiency is known as a low level of folic acid in the body it is involved in DNA synthesis. deficiency of folate in pregnant women due to increase in demand and when the dietary intake of folate is inadequate and when the body excrete or lose more than usual , some medications interfere with the body ability to use folate which lead to folate deficiency.

During pregnancy the body need extra folate to produce normal red blood cells . (Hoffbrand and Weir, 2001).

vitamin B-12, also called cobalamin, is a water-soluble vitamin, It is normally involved in DNA synthesis and regulation. The body need vitamin B-12 to form a healthy red blood cells and when pregnant women doesn't get enough vitamin B-12 from diet her body cannot produce healthy red blood cells. Vitamin B-12 is found in most animal derived foods, including fish and shellfish, meat (especially liver), poultry, eggs, milk, and milk products. (Killen *and* Brenninger, 2013) .

## **1.2 Literature Review.**

### **1.2.1 Blood**

Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transport metabolic waste products away from those same cells. The blood composed of cells and plasma, the blood cells are mainly red blood cells (RBCs), white blood cells (WBCs), and platelets. The most abundant cells in blood are red blood cells. White blood cells have immunological function, and platelets play an important role in coagulation. Plasma which constitutes 55% of blood fluid, is mostly water and contains proteins, glucose, mineral ions, hormones. Plasma being the main medium for excretory product transportation. (Alberts *et al.*, 2012).

#### **1.2.1.1 Blood contents**

Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature blood cells lack nucleus and organelles in mammals. The proportion of blood occupied by RBCs is referred to as hematocrit and is normally about 45%. White blood cells are a part of the body immune system they destroy or remove old or aberrant cells and cellular debris, as well as attack infectious agents and foreign substances. The cancer of leukocyte is called leukemia. Thrombocyte also called platelets, they take part in blood coagulation. Fibrin from coagulation cascade create a mesh over the platelet plug. (Ganong, 2003).

Plasma is about 55% of blood content, it is the bloods liquid medium which by itself is straw-yellow in color. The blood plasma volume of 2.5-3.0 L in an average human, It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma



circulate dissolved nutrients, such as glucose, amino acids, and fatty acids or bound to plasma proteins, and remove waste products such as carbon dioxide, urea, and lactic acid. Other important components of blood include blood clotting factors which facilitate coagulation, and immunoglobulins and various electrolytes mainly sodium and chloride. (Ganong, 2003).

### **1.2.1.2 Blood pH**

Blood pH is regulated to stay within the narrow range of 7.35-7.45, making it slightly basic. Blood that has a pH below 7.35 is too acidic, whereas blood that is above 7.45 is too basic. Blood pH, partial pressure of oxygen ( $pO_2$ ), partial pressure of carbon dioxide ( $pCO_2$ ), and  $HCO_3^-$  are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through the respiratory system and urinary system in order to control the acid-base balance and respiration. An arterial blood gas test will measure these. (Waugh *et al.*, 2007)

### **1.2.2 Hemoglobin function and synthesis :**

The major function of hemoglobin is to carry oxygen ( $O_2$ ) from lung to the tissues and return carbon dioxide ( $CO_2$ ) from tissues to the lung.

The hemoglobin begins to be produced during the pro-erythroblast stage to the reticulocyte in the bone marrow of the RBC cycle, the synthesis takes place in mitochondria and ribosomes by a series of biochemical reactions. Protoporphyrin combined with iron to form heme. Then heme exits the mitochondria and combines with globin molecules which is synthesized in ribosomes. The synthesis of heme is a complex process that involves multiple enzymatic steps. The process begins in the mitochondrion with the condensation of succinyl-CoA and glycine to form 5-aminolevulinic acid. A series of steps in

the cytoplasm produce coproporphrynogen III, which re-enters the mitochondrion. The final enzymatic steps produce heme. (Bayens and Stipanuk, 2000).

**Table (1.1) : Human hemoglobin (Maton, 1993)**

<b>Human Hemoglobins</b>		
<b>Embryonic hemoglobins</b>	<b>Fetal hemoglobin</b>	<b>Adult hemoglobins</b>
Gower 1- zeta(2), epsilon(2) Gower 2- alpha(2), epsilon (2) Portland- zeta(2), gamma (2)	hemoglobin F- alpha(2), gamma(2)	hemoglobin A- alpha(2), beta(2) hemoglobin A2- alpha(2), delta(2)

### **1.2.3 Anemia**

Anemia is strictly defined as a decrease in red blood cells (RBCs) mass and hemoglobin according to the age and sex. It can be classified according to the cause, by the defective production of red cells, or and increased rate of loss of cells; either by bleeding or hemolysis. The causes of defective production of red blood cells include : deficiency of iron, vitamin B12, and folate, or due to primary disease of the bone marrow . Hemolytic anemia causes are genetic which include: membrane defect, hemoglobin disorder and enzyme deficiency, and acquired hemolytic anemia include immune and non-immune disorders. It can also be classified according to the size of the red cells as microcytic, normocytic and macrocytic anemia.(Maakaron *et al.*, 2015)

Laboratory investigations play an essential part in diagnosing anemia, establishing its etiology and determining its appropriate treatment. Investigation includes hemoglobin level, red cell count, mean cell volume (MCV), packed cell volume (PCV) and red cell indices (MCH) and (MCHC), and reticulocyte count. Bone marrow aspiration and hemoglobin electrophoresis are necessary for investigation of anemia. (Campbell, 2005).

### **1.2.3.1 Classification of Anemia**

Anemia can be classified morphologically based on the size of the cells and the hemoglobin concentration into: Macrocytic anemia, normochromic normocytic anemia, hypochromic microcytic anemia. Anemia's may also be classified functionally into : hypo-proliferative when there is proliferation defect, due to decrease level of erythropoietin or lack of iron or marrow damage. or ineffective when there is a maturation defect due to B-12 deficiency, folate deficiency or stromal disease (myelofibrosis), or due to non-sideroblastic anemia (iron deficiency) or sideroblastic anemia due to globin abnormality. Or due to hemolysis when there is a survival defect, due to autoimmune hemolysis or RBCs membrane defect such as: hereditary spherocytosis or due to enzyme deficiency such as: G6PD deficiency and pyruvate kinase deficiency or due to hemoglobin synthesis defect such as: Sickle cell anemia and thalassemia. (Maakaron *et al.*, 2015)

### **1.2.4 Pregnancy**

Pregnancy is defined as the period from conception to birth. After the egg is fertilized by a sperm and then implanted in the lining of uterus, it develops into the placenta and embryo, and later into a fetus. Pregnancy usually lasts

40 weeks beginning from the first day from the woman's last menstrual period and is divided into three trimesters each lasting three months.

By the end of the first trimester the major blood vessels and the roof of the mouth are almost completed, as the face starts to take on a more recognizably human appearance. Fingers and toes appears. All major organs are now beginning to form; the kidneys are now functional and the four chambers of the heart are now complete. (Hurt *et al.*, 2012).

By the end of the second trimester the lungs are not fully developed. The fetus experiences rapid growth as its internal organs continue to grow at this point, the mother may feel her fetus move and she can hear the heart beat with stethoscope. Weighing 450-680g .

In the third trimester the fetus drops lower into the mother's abdomen and prepares for the onset of labor, which may begin anytime between the 37<sup>th</sup> and 42<sup>nd</sup> week of gestation. Most healthy babies will weigh 2.7-4.0 kg at birth.(Hurt *et al.*, 2012)

#### **1.2.4.1 Anemia in Pregnancy**

With normal pregnancy blood volume increases which result in a hemodilution. Although red blood cells (RBCs) mass increases during pregnancy, plasma volume increases more resulting in relative anemia . This result in a physiologically lower hemoglobin level (Hb) , PCV and RBCs count, but it has no effect on MCV Causes of anemia during pregnancy are multi-factorial this include, Nutritional deficiencies such as iron, folate, vitamin B12 which result in megaloblastic anemia and parasitic diseases such as: malaria and hook worms. Also fluid overload (hypervolemia) cause decrease hemoglobin concentration and apparent anemia. Also maybe due to

pre-existing condition of thalassemia and sickle cell anemia. However iron deficiency is believed to be the main underlying cause for anemia in pregnancy due to increase in demand of iron for fetal and maternal tissue growth.(VanderJagt *et al.*, 2007).

### **1.2.5 Neonatal Anemia**

Newborn babies do not start to make new RBCs until they are about one month old, as some of the older RBCs start to breakdown which are gained from their mother, the baby may not have enough red blood cell to replace them from the first 2 or 3 months of life. For every new born baby this causes a mild type of anemia called physiologic or normal anemia. Once a baby starts making new RBCs the red cell count gradually goes back to normal .

The commonest causes of neonatal anemia include: obstetrical cause; as placenta abruption, trauma to placenta. RBCs destruction due to intrinsic causes, hereditary RBCs disorder or extrinsic causes due to immune hemolysis as Rh and ABO incompatibility and slow RBCs production due to lack of iron or other nutrient in baby blood . (Scholl *et al.*, 1992)

### **1.2.6 Iron deficiency anemia**

Iron deficiency is the most common cause of anemia due to many reasons, the person might become deficient in iron these include; inadequate iron intake, malabsorption and because iron is essential during times of rapid growth and development, pregnant women and young children may need even more iron-rich foods in their diet.

Pregnancy or blood loss due to menstruation in women of child-bearing age, internal bleeding such as: stomach ulcer or colon cancer or inability to absorb iron due to celiac disease or intestinal surgery.

Stages of ID can be explained by excessive depletion of iron from storage tissues to transport proteins and finally manifesting as iron deficiency anemia (IDA). (Nemeth and Ganz, 2006).

### **1.2.7 Iron absorption**

Iron is mainly absorbed in the duodenum and upper jejunum of small intestine. A protein called divalent metal transporter one (DMT1) facilitates iron transfers across intestinal epithelial cells ( Mckie *et al.*, 1998 ).

Heme iron (animal food iron) typically absorbed at rate 7—35% which derived from HB and myoglobin present in animal tissues, such as meat, sea food and poultry. Non-heme iron (plant food iron) typically absorbed at rate of ( 2—20%), which found in leafy green vegetables, nuts, oil seeds. (Baynes and Stipanuk, 2000). Normally individuals absorbed about 10% of dietary iron or 1-2 mg per day .Most of absorbed iron is used by erythropoiesis and all body cells need iron . It is crucial for oxygen transport, energy production, cellular growth and proliferation. the human body contain an average of (3.5 g) of iron (male 4 g , female 3 g ). Iron homeostasis is closely regulated via intestinal absorption. The central regulator of iron homeostasis is hepcidin , a 25 amino acid peptide expressed and secreted by hepatocytes . In turn hepcidin synthesis is increased by iron over loading and decreased by anemia and hypoxia. Hepcidin is markedly induced during inflammation, trapping iron in macrophages, decreasing plasma iron concentrations, and contributing to the anemia of inflammation. Hepcidin deficiency due to the dysregulation

of its synthesis causes most known forms of hemochromatosis. (Nemeth and Ganz, 2006).

### **1.2.8 Iron transport :**

Most absorbed iron is transported in the blood stream bound to glycoprotein transferrin which is a  $\beta$ -globulin with approximate of MW 80 KDa and is synthesized in the liver. Transferrin is a carrier protein that play a central role in regulating the transport of iron from the side of absorption to virtually all tissues and plays a key role in area where erythropoiesis and active cells division occur. Each transferring molecule has the ability to carry two iron ions in the ferric form ( $\text{Fe}^{+3}$ ). Increase plasma transferrin level is often seen in iron deficiency anemia, a decreased plasma transferrin can occur in iron overload diseases, absence of transferrin result from a rare genetic disorder known as atransferrinemia (Cook, *et al.*, 1993).

### **1.2.9 Iron Storage :**

Iron initially stored in ferritin molecules , a single ferritin molecules can store up to 4000 iron atoms when excess iron dietary is absorbed the body respond by producing more ferritin to facilitate iron storage. Iron is stored in ferritin complexes that are present in all cells but most common in bone marrow , liver and spleen . iron also stored as a pigment called hemosiderin contain ferric oxide ( rust ) . Low level of ferritin indicate that there's a risk for lack of iron which could lead to anemia. In the other hand if serum ferritin is high there's iron in excess or there's acute inflammatory reaction . a normal C. Reactive Protein can be used to exclude elevated ferritin caused by acute phase reaction ( Heinrich *et al.*, 1977).

### **1.2.10 Regulation of iron transfer to the fetus :**

Iron transfer from mother to the fetus across the placenta and subsequently transfer into the fetal circulation . The first step in transfer of iron across the placenta involves transferrin binding to its receptor (TfR ), on the placenta microvillar surface . after binding is completed the complex is incorporated into clathrin – coated vesicles and internalized . The pH inside the vesicle is reduced , the iron is released from the transferrin. Inside the vesicle , iron ( $\text{Fe}^{+2}$  ) moves through a channel known as divalent metal transporter I (DMT1) into the cytoplasm ( McArdle *et al.*, 2008 ).

How it gets transferred to the fetal side of the cell is not known . when maternal iron reduced the number of placental TfR increases which reflect in more iron taken up by placenta . Excessive iron transport to the fetus may be prevented by placental synthesis of ferritin ( Allen, 2000 ) .

### **1.2.11 Serum Ferritin :**

#### **1.2.11.1 Ferritin Molecule :**

Ferritin is an ubiquitous intracellular protein that store iron and releases it in a controlled fashion . Ferritin consist of 24 protein subunits and serves to store iron in non-toxic form. Ferritin that is not combined with iron is called apoferritin . Apoferritin bind to free ferrous iron and store it in a ferric state. iron ferritin can be extracted for release by the RES cells. Under steady state conditions the serum ferritin level correlates with total iron stores, thus the serum ferritin is the most convenient laboratory test to estimate iron stores .

Low level of ferritin indicate there is a risk for lack of iron which could lead to anemia , also low serum ferritin may indicate hypothyroidism and vitamin



C deficiency . Excess of serum ferritin indicate high level of iron or there is acute inflammatory reaction in which ferritin is mobilized without iron excess . a normal C. Reactive protein can be used to exclude elevated ferritin which cause by acute phase reaction . in vertebrates serum ferritin is usually found within cells. Although it is also present in small quantity in plasma (Punnonen *et al.*, 1997).

**Table (1.2): normal ferritin blood level (Punnonen *et al.*, 1997)**

<b>Women</b>	12-150 ng/mL
<b>Neonates</b>	25-200 ng/mL

### **1.2.12 Anemia Investigations**

Laboratory investigations play an essential part in diagnosing anemia, establishing its etiology and determining its appropriate treatment. Investigation includes hemoglobin level, red cell count, mean cell volume (MCV), packed cell volume (PCV) and red cell indices (MCH) and (MCHC), and reticulocyte count. Bone marrow aspiration and hemoglobin electrophoresis are necessary for investigation of anemia (Campbell, 2005).

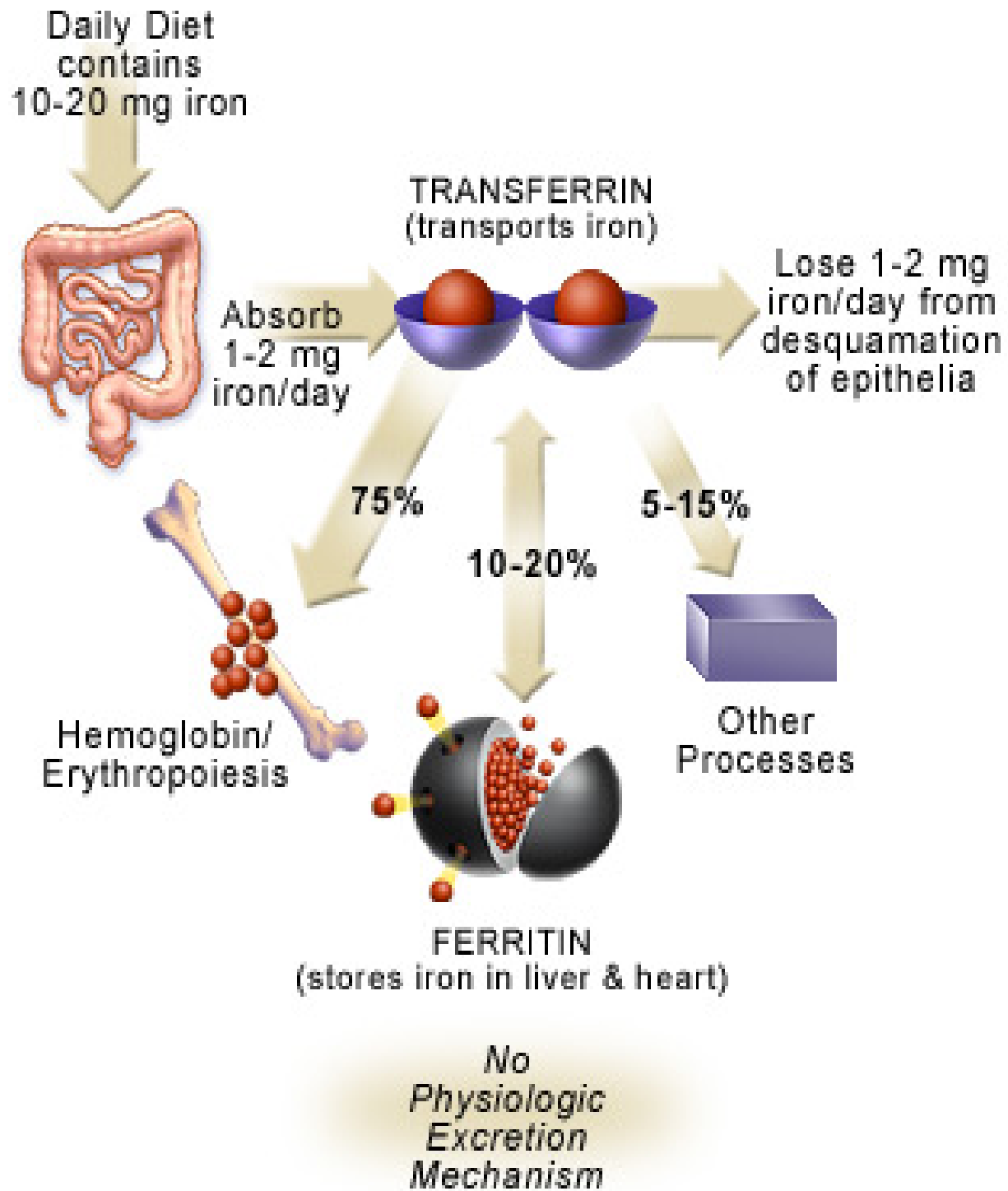


Figure 1.1: Iron absorption and storage in the human body (Pynaert *et al.*, 2007).

### 1.2.13 Previous studies :

The prevalence of anemia among the pregnant women ages 21 – 43 years in Sudan was 34.2% . The prevalence of anemia among non-pregnant women in Sudan ages 15– 49 years was 31.2% ( WHO, Geneva, 2011 ).

A study from Sudan identified iron deficiency anemia, malaria and hookworms as a major cause of anemia among pregnant women. Investigated in 744 pregnant Sudanese women attending the antenatal clinic of Newhalfa teaching hospital, Eastern Sudan between 2003-2004 of those 466 (62.6%) had anemia; hemoglobin less than 11.0 (g/dl) 52.4% had mild anemia (Hb) .. 9-10 (g/dl) 8.1% had a moderate anemia (Hb) 7-8.9 (g/dl) 2.2% had a severe anemia (Hb) less than 7.0 (g/dl). Iron status during pregnancy was conducted in Gezira , central Sudan faculty of medicine university of Gezira between 2010—2011 among pregnant women in late third trimester presenting for Wadmadani teaching hospital 600 ladies investigated the study showed low serum iron and serum ferritin in most pregnant women (22.13 ng/mL  $\pm$  10 ) (Sudanese journal of public health V.1.) .

In the screening of iron deficiency anemia (ID) WHO reported that individual with more marked anemia usually have ferritin  $\leq$  60  $\mu$ g/dl . Many studies done in many countries for level of iron and serum ferritin , study in Saudi Arabia demonstrated that iron deficiency was significantly associated with those who are in the third trimester 31.9%.( Al-Buhairan *et al.*, 2001 ). A study in Singapore demonstrated that ID anemia is the common cause of anemia in pregnancy 29% .(WHO, Geneva, 2011 ). In Pakistan, a study to investigate the relationship between maternal anemia and prenatal outcome in a cohort of 629 pregnant women was performed , a total of 313 - 50.8% (Lone *et al.*, 2004). In Iran a study was performed on a sample of a total of 70

pregnant women to assess hemoglobin and serum ferritin levels in newborn of anemic mother to determine the relationship between maternal iron status and their newborns , the study showed that maternal Hb concentration had influence on neonatal parameters (Hadipour *et al.*, 2010), in Brazil , a cross-sectional study on a sample 95 pregnant women and their umbilical cord to determine the relationship between iron nutritional status of pregnant women and their newborns , a study showed significant association between neonatal and maternal parameters ( Paiva *et al.*, 2007).

Causes of anemia other than nutrient deficiency include malaria, intestinal parasites ( hookworm ) and genetically determined hemoglobinopathies such as sickle cell anemia and thalassemia. Important point is to differentiate between hypochromic , microcytic anemia due to iron deficiency and one due to  $\beta$ -thalassemia trait : assay of serum ferritin may be useful here because patients with uncomplicated  $\beta$ -thalassemia trait usually have a normal or increased serum ferritin level for those anemic but not due to iron deficiency . ( Loria *et al.*, 1978). Study in Sudan conducted by (Elgari, 2013), the result showed that there were significant decrease in hemoglobin of pregnant women compared to non pregnant women (P value <0.05). A study done by (Farah and Mansoor, 2012) showed there was statistically significant differences in hemoglobin and S.ferritin level among anemic pregnant women compared to non anemic pregnant women (P value <0.05). Study done by (Abdelgader *et al.*, 2014) which aim to measure hemoglobin level , RBC indices and iron status in pregnant women in sudan , the results showed that 10 % had low Hb level and 22.5% had low MCV.

### **1.3 Rationale :**

A better understanding of maternal anemia in pregnant women and its effects on newborns. Hemoglobin and serum ferritin concentration might provide useful information on treatment programs and antenatal care during pregnancy. To assess the hemoglobin and serum ferritin of anemic and non-anemic women and their newborn which reflect the association between hemoglobin and serum ferritin level in mothers and their newborns.

## **1.4 Objectives**

### **1.4.1 General objective :**

Determination of hemoglobin and serum ferritin concentration in pregnant women and their newborns of anemic and non-anemic pregnant women.

### **1.4.2 Specific objectives :**

- To measure hemoglobin and serum ferritin in pregnant women.
- To measure hemoglobin and serum ferritin in neonates of anemic and non-anemic women.
- To associate a hemoglobin level and serum ferritin in pregnant women with their newborns.
- Determination of Hemoglobin and serum ferritin level in anemic pregnant women and non-anemic pregnant women and their newborns associate with iron supplementary.

# **Chapter two**

## Materials and Methods

## **2 Materials and Methods**

### **2.1 Study Design:**

This is a case control prospective study. Was conducted in Khartoum state , locality of maternity center and hospitals . In the period April to August 2015.

### **2.2 Sample size:**

The sample size was Included 70 anemic pregnant women (Hb <11 g/dl) as case group and 70 non-anemic pregnant women as control group (Hb  $\geq$  11 g/dl) as defined by WHO and their new born babies

### **2.3 Inclusion Criteria:**

All pregnant women at age (23 to 41 years) booked at the late of third trimester presenting to maternity centers for antenatal care with no medical condition and not receiving blood transfusion were included in this study.

### **2.4 Exclusion Criteria:**

Exclusion criteria includes pregnant women in the first and second trimester and those have chronic diseases such as hepatitis , HIV infection and those had a blood transfusion were excluded from this study

### **2.5 Data Collection:**

The data was collected by using laboratory procedures and information obtained from case and control pregnant women by check list and questioner . the questionnaire (anex1) was based on personal clinical history data all interviews were conducted face to face by one technologist who had bachelor degree of medical technology



## 2.6 Sample Collection :

Three milliliters of blood was collected from each women before labor and two milliliters was collected from the blood of their newborns into ethylenediamine tetra acetic acid (EDTA) bottle for blood count analysis (CBC).

Another 3 ml of blood was collected from each women before labor and 2 ml of blood was collected from their newborns into plain bottle for serum ferritin assay.

## 2.7 Materials :

### 2.7.1 Chemicals and Reagents:

Chemicals and reagents used in this study are shown in the below.

Reagents	Supplier
HBG	Sysmex corporation –Japan
Ferritin	Roche Diagnostic International – Switzerland
Controls Materials	
HBG controls	Sysmex corporation -Japan
Multi-controls sera	Roche Diagnostic International - Switzerland Sysmex corporation - Japan

## 2.7.2 Equipments:

The main equipments used in this study are listed in the blow.

Instrument	Manufacturer
Sysmex Kx-21 N	Sysmex corporation -Japan
Cobas 411 e	Sysmex corporation -Japan
Centrifuge	Hetich – Germany
refrigerator	L-G Korea
Freezer -20 C	L-G Korea

## 2.8 Hemoglobin methods :

Hemoglobin was measured using Sysmex Kx-21 N ,.

### 2.8.1 Principle detection :

Non- cyanide method (HGB). particles can be counted and sized either by electrical impedance or by light scattering. Automated instruments have at least two channels. In one channel a diluents is added and red cells are counted and sized. In another channel a lytic agent is added, together with diluents, to reduce red cells to stroma, leaving the white cells intact for counting and also producing a solution in which Hb can be measured

### 2.8.2 Procedure :

1. The reagent needed was checked for validity, availability and completion.
2. The power switch was turned on . self auto rinse, and background check was automatically performed and the vend (vend for analysis ) was appeared.

3. Whole blood mode was selected. Sample number : inputted by pressing sample number then number of sample was entered.
4. Enter key was pressed. Sample was mixed sufficiently. The tube was sated to sample probe, and in that condition the start switch was pressed. When the LCD screen display analyzing the tube was removed.
5. After that the unit executes automatic analysis and the result was displayed in LCD screen.
6. The result was printed out. ( Built in thermal printer ).

### 2.8.3 Quality Control:

Quality control was performed to ensure accuracy and precision of the instrument (Sysmex KX- 21 N), by using three controls samples obtained from manufacturer, High control, Low control and Normal control which fall within the established confidence limit. ( table 2.1 ).

Table ( 2.1) Result of hemoglobin controls (reference values)

Control	Confidence limits	Result
Low control	5.8 – 6.4 g/dl	6.1g/dl
Normal control	12.3 – 13.1g/dl	12.8g/dl
High control	16.3 – 17.5g/dl	16.7g/dl

Table (2.2): Normal range of hemoglobin (Campbell, 2005)

Female	( 12.5 – 16 g/dl )
Newborn	( 13.0 -- 17 g/dl )

## 2.9 Measure of Serum Ferritin :

Serum ferritin was measured using Cobas 411 e instrument .

### 2.9.1 Principle:

Sandwich assay Ferritin of sample with biotinylated ferritin monoclonal-specific antibody and monoclonal ferritin – specific antibody labeled with ruyhenium complex form a sandwich complex in assay cup an d transported in a measuring cell.

### 2.9.2.Procedure

1. Once analyzer is switch on, the initialization process start. During initialization the mechanisms are reset to their home position .
2. The reagent needed was checked for validity, availability and completion.
3. In the first step 10 micro liter of sample ,a biotinylated monoclonal ferritin-specific antibody and monoclonal ferritin –specific antibody labeled with a ruthenium complex in assay cup form a sandwich complex.
4. Incubation 9 minutes (antibodies capture the ferritin present in the sample ).
5. Streptavidin –coated paramagnetic microbeads are added .

6. Incubation 9 minutes, ( complex becomes bound to the solid phase via interaction of biotin and streptavidin ).
7. The content of the assay cup is aspirated into the measuring cell where the microparticles are magnetically capture onto the surface of the electrode.
8. Wash buffer were added to the assay cup and aspirated , unbound substance are then removed .
9. Application of a voltage to the electrode induce chemiluminescent emission which is measured a photomultiplier. The amount of light produce is directly proportional to the amount of ferritin in the sample.

### **2.9.3 Quality Control of Ferritin:**

Individual control available from manufacture were run with each assay and fall within established confidence limits.

### **2.10 Ethical Consideration**

Administrations of maternity centers which is included in the study have been informed with the goal and the aim of the study to get their approval in order to be conducted. Consent was obtained from pregnant women by themselves, their mothers or their husbands.

## **2.11 Statistical Analysis :**

The collected was analyzed using the computer program SPSS statistics version 15.1.1 ( Statistical Package for the Social Science) , later modified to read Statistical Product and Service Solutions . P significant level was set at  $P \leq 0.05$ .

# **Chapter Three**

## Results

## 3 Results

### 3.1 Demographic Data:

Age of the case group matched the control group in age , showed minimum of 21 years and maximum of 42 years (mean=29.3years)

Gestational age of the studied mothers cases and control was in the third trimester.

Age distribution of the study population case and control, there were 44 women aged between 20-25 years, 54 aged between 26-30 years, 28 between 31-35 years, 10 between 36-40 years, 4 between 41-45 years. (Table 3.1)

Table 3.2 shows a total of 59.3% of the pregnant women included in the study of ( case & control )were on iron supplements during pregnancy.

30% of case group received iron supplements during pregnancy while 70% did not administer any iron supplements during their pregnancy .

88.6% of the non anemic group control received iron supplements during pregnancy while 11.4 did not administer any iron supplements during pregnancy

Result showed control group who received iron supplement during pregnancy their hemoglobin and serum ferritin levels were significantly higher than cases who received iron supplements during pregnancy.(P value = 0.001).



### 3.2 Laboratory Data

Table (3.3) pointed to hemoglobin level and serum ferritin of anemic and non-anemic mothers.

The result showed that the mean of hemoglobin of the case was  $9.3 \pm 0.72$  g/dl and that of control was  $11.6 \text{g/dL} \pm 0.63$ . Comparison of the mean values for hemoglobin of cases and controls groups showed significant differences (P value = 0.001)

The result showed that the average of ferritin of cases was  $7.8 \pm 8.6$  ng/ml and average of serum ferritin of controls was  $14.31 \pm 9.32$  ng/ml. Comparison of the mean values for ferritin of cases and controls groups showed significant difference (P value = 0.001).

Table (3.4): Shows the hemoglobin and serum ferritin levels of newborns of anemic and non-anemic mother groups.

The mean level of hemoglobin result for newborns of anemic mothers was  $14.53 \text{ gm/dl} \pm 1.3$

The mean value of hemoglobin for newborns of control mothers was  $15.43 \text{ g/dL} \pm 1.2$

The result showed significant differences between cases and controls in the mean values of Hb ( $14.53 \pm 1.34$  Vs.  $15.43 \pm 1.22$  g/dl) (P value = 0.039).

The study showed the Hb level of newborns is dependent on mothers Hb level.

The mean value of ferritin for newborns of anemic mothers was  $72.4 \pm 27.5$  ng/ml and the mean value of ferritin for newborn of non-anemic mother was  $96.7 \pm 29.3$  ng/ml , the result showed significant differences between cases and controls In the mean value of ferritin (  $72.4 \pm 27.5$  Vs.  $96.37 \pm 29.3$ ) ng/ml. (P value = 0.001).

The study showed that ferritin level of newborns is dependent on the mothers ferritin level .

Table (3.1):Age Distribution of study population

Age	Anemic	Non-anemic	Total No.
20-25 years	21	23	44
26-30 years	25	29	54
31-35 years	16	12	28
36-40 years	6	4	10
41-45 years	2	2	4

Table (3.2): The iron supplements of case and control groups.

Variable	Anemic mothers		Non-anemic mothers		P value
	Received iron	Not received iron	Received iron	Not received iron	
No.	21	49	62	8	0.001
Percent	30%	70%	88.6%	11.4%	

Table (3.3): Mean of Hemoglobin level and serum ferritin of anemic mothers (case) and non-anemic mothers (control) groups.

Variable	Cases (n=70)		Controls (n=70)		P value
	mean	SD	Mean	SD	
Hb (g/dL)	9.3g/dL	±0.72 SD	11.6 g/dL	±0.63 SD	0.001
Serum Ferritin ng/ml	7.8 ng/mL	±8.6 SD	14.31 ng/mL	±9.32 SD	0.001

Table (3.4): Mean level of Hb and serum ferritin of newborns of anemic and non-anemic mothers

Variable	Cases (n=70)		Controls (n=70)		P value
	mean	SD	Mean	SD	
HB g/dl	14.53 g/dL	1.34 SD	15.43 g/dL	1.22 SD	0.039
Ferritin ng/ml	72.4 ng/mL	27.5 SD	96.7 g/dL	29.3 SD	0.001

# **Chapter Four**

**Discussion,**

**Conclusion**

**and**

**Recommendations**

## 4 Discussion, Conclusion and Recommendations

### 4.1 Discussion

The risk of anemia is particularly high in pregnant women. Iron transfer from mother to fetus, maternal iron is the only source of fetal iron.

Therefore the maternal iron status will affect the iron status of neonates.

In this study the result in newborns from anemic mother showed significant relationship between neonatal hemoglobin and serum ferritin as compared with newborns of non-anemic mothers.

It is interesting that the newborns to anemic mothers (cases) had low mean level of hemoglobin  $14.5 \text{ g/dl} \pm 1.34$  compared to the mean hemoglobin level of newborn to non-anemic mothers (controls)  $15.43 \text{ g/dl} \pm 1.22$  (P value = 0.039), and the mean value of serum ferritin for newborns of anemic mothers (cases) was  $72.4 \text{ ng/ml} \pm 27.5$  compared to the mean value of serum ferritin for newborns of non-anemic mothers (controls)  $96.7 \text{ ng/ml} \pm 29.3$  (P value = 0.001)

The study showed that the mean hemoglobin and the serum ferritin of newborns were significantly different between non-anemic (control) and anemic (case) groups.

This study agrees with (Elgari, 2013) study, concerning the level of hemoglobin of pregnant women (P value <0.05).

The study also agrees with a study done by (Farah and Mansoor, 2012) showed there was statistically significant differences in hemoglobin and S.ferritin level among anemic pregnant women compared to non anemic pregnant women (P value <0.05).

This study agree with the (Singla *et.al* 1996) study that showed the level of hemoglobin and serum ferritin were significantly low in the newborn blood of anemic women than non-anemic women. (Singla *et.al* 1996).

The result also agree with (Harthoorn, *et al.*, 2001) who found that serum ferritin was higher in newborn blood than in respective maternal sample but he also stated that serum ferritin of newborn delivered by mothers with very low serum ferritin concentration 35 lower than in newborns of mother having normal ferritin level. (Harthoorn, *et al.*, 2001)

The result of this study showed a correlation between neonatal hemoglobin and serum ferritin, and maternal hemoglobin and serum ferritin, which is similar with (Emery and Barry, 2004). According to other studies , maternal anemia is an important risk factor that can cause anemia during infancy, even if there is no differences in hemoglobin or serum ferritin are seen in newborns at birth (Kumar *et al.*, 2008).

This study also agrees with a study performed in Iran that showed that maternal Hb and serum ferritin concentration had influence on neonatal parameters. (Hadipour *et al.*, 2010)

However the total iron requirement during pregnancy increase due to the requirement of the fetus . the daily requirement during the third trimester is 6 mg. (Nuchprayoon *et at.*, 2002)

## 4.2 Conclusion

- There are association between maternal hemoglobin and serum ferritin with their neonates hemoglobin and serum ferritin .
- The mean hemoglobin in newborns from anemic mothers (cases) is lower than the mean hemoglobin levels of newborns from non-anemic mothers (control).
- The mean serum ferritin of newborns from anemic mothers (cases) is lower than the mean levels of serum ferritin of newborns from non-anemic mothers (control).
- There was high significant association between the percentage of anemic and non-anemic women who received iron supplement regularly.



### **4.3 Recommendations:**

1. Other research studies are needed to establish the normal values indices in normal pregnant women and their babies.
2. Regular investigation of pregnant women for Hb and serum ferritin.
3. Administration of regular iron supplementation during pregnancy .
4. Nutritional education program to improve the dietary intake of pregnant mothers.
5. Continuous medical education program for the medical staff concerning anemia of pregnancy.

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# Appendix

## Annex 1

بسم الله الرحمن الرحيم

استبيان:

كل الشكر و التقدير للذين تعاونوا معنا في انجاز بحث بعنوان .. مستوى الهيموجلوبين و الفريتين لدى النساء الحوامل و مواليدهم الجدد من امهات مصابات بالانيميا و امهات غير مصابات بالانيميا في ولاية الخرطوم , علما بان كل المعلومات في هذا الاستبيان سرية جدا .

و شكرا..

-عمر الام الحامل ..... سنة

-هل تتناولين اقراص الحديد اثناء الحمل ؟ نعم  لا

-هل تتناولين الوجبات الغذائية بانتظام ؟ نعم  لا

-ماهي نسبة هيموجلوبين الام اثناء الحمل ..... جرام/ديسيلتر