Sudan University of Science and Technology
College of Graduate Studies

# Detection of Iron Deficiency Anemia among Female in Reproductive Age in Khartoum State(2017)

# الكشف عن فقر الدم بنقص الحديد وسط النساء في عمر الإنجاب بولاية الخرطوم (2017م)

A Dissertation Submitted in Partial Fulfillment for the Requirements of the Degree Of M.Sc. In Medical Laboratory Science (Hematology and Immunohematology).

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# الآيـــــــــــة

**بسم الله الرحمن الرحيم**

**قال تعالى:**

((يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا العِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ))

**صدق الله العظيم**

**المجادلة (11)**

# *DEDICATION*

*I dedicate this work to:*

*My darling parents who are supporting me and encouraging me to success.*

*My husband and my kids and my sisters.*

*My friend and colleagues who gave me the possibility of completing this dissertation*

*Everyone who has helped me to learn new things and to reach this level.*

*Riham*

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## Abstract

This was descriptive cross-sectional study, aimed to investigate status of iron deficiency anemia among Sudanese women in reproductive age (15-47) years .It was carried out during the period of (January –April 2017) at saad Abo Ellele Hospital. The study included 100 Sudanese females; 70 non pregnant women and 30 pregnant women, their age ranged 15-47years.

All subjects were verbally informed about the study and their consent for participation was obtained. Venous blood sample of 5ml was collected from each subject. Automated hematological analyzer (Sysmex KX21N) was used for CBC and automated biochemical analyzer (Selectra XL) was used for serum iron and total iron binding capacity.

The data collected consist of demographic data of women included menstrual status, age, pregnancy status; dietary, medical history and educational level.70% of women were normal. The result was analyzed by Statistical Package for Social Sciences (SPSS version 11.5). Age divided into three age groups (15-25) years, (26-36) years and (37-47) years with mean and STD (24.7$\pm$5.6).

The prevalence of anemia (Hb <11.0g/dl) was27% among the study group and the majority of women had moderate anemia (55.6%). (14.8 %) were mild anemic, while severe anemic was (29.6%). The study observed that there was statistically significance difference in the mean RBCs count, PLT scount, Hb level, MCV, MCH, and MCHC, Serum iron and TIBC among anemic group when compared with non-anemic group ($P<0.05$).

Result also show that there was statistically insignificant difference in measured parameters (serum iron, TIBC and Hb) between pregnant and non-pregnant women (*P. values* 0.27, 0.39 and 0.10) respectively.

Also there was no significant association between severity of iron deficiency anemia and demographic data ($P>0.05$).addition, there was statistically

significance difference in iron store status between different group of Iron intake (*P* .value =0.01). This high prevalence of iron deficiency anemia among women may be due to malnutrition and lack of medication during pregnancy.

## مستخلص الدراسة

هذه الدراسة وصفية مقطعية هدفت الى تحديد حالات فقر الدم بسبب نقص الحديد بين السيدات السودانيات في سن الإنجاب. وقد أجريت هذه الدراسة خلال الفترة من شهر يناير الى ابريل 2017 بمستشفى سعد أبو العلا التعليمي. شملت الدراسة 100أمراة سودانية، 70 منهن غير حوامل و30منهن حوامل (أعمارهن بين16-47 سنه). وتم إخطارهن بهذه الدراسة وأهميتها وأخذت موافقتهن علي المشاركة. وقد تم جمع 5مل من الدم الوريدي من كل أمراة في الدراسة. وتم استخدام جهاز التحليل الدموي الالي ( System Kx21N)لإجراء فحوصات تعداد الدم الكامل ثم استخدام جهاز التحليل الكيميائي الالي ( Selectra xl)لإجراء فحوصات الدم الكيميائية لمعرفة مستوي الحديد والسعة الكاملة لارتباط الحديد.

جمعت البيانات الديموغرافية (السكانية) عن طريق استبيان وتضم الحمل، الغذاء، التاريخ الطبي، المستوي التعليمي والعمر.70% من النساء وجدن سليمات. وقد تم تحليل النتائج إحصائيا بإستخدام برنامج الحزم الإحصائية الإصدا(11.5). و تم تقسيم المشتركين في الدراسة علي حسب الأعمار الي ثلاث فئات(15-25)، (26-35)، (36-47) سنة وكان المتوسط الاعمار(24.7 $\pm$5.6) أظهرت النتائج ان 27%من الحالات التي شملتها الدراسة كانت تعاني من فقر الدم، معظمهن بين حالات متوسطة (55.6%)، وبسيطة(14.8%) و(29.6%) تعاني من فقر الدم الشديد. كما بينت الدراسة ان هناك اختلاف ذو دلالة إحصائية واحتمالية اقل من (0.05) في متوسطات عدد كريات الدم الحمراء ,مكداس الدم الأحمر,مؤشرات الخلية الحمراء(متوسط حجم الحلية ومتوسط هيموغلوبين الخلية)، مستوي الحديد في مصل الدم والسعة الكاملة لارتباط الحديد.وأوضحت الدراسة انه لا يوجد فرق ذو دلالة إحصائية عند قياس (نسبة الحديد في مصل الدم,والسعة الكاملة لارتباط الحديد والهيموغلوبين) عند المقارنة بين الحوامل وغير الحوامل(0.10، 0.27،0.39) بتتابع.

ختاما أشارت النتائج الي وجود إختلاف ذو دلالة إحصائيه عند مقارنة مخزون الحديد في الجسم مع الحديد المتناول في الغذاء (القيمة المعنوية0.01).ان هذا الانتشار المرتفع لفقر الدم بين النساء ربما بسبب سوء التغذية وعدم تناول الحديد اثناء فترة الحمل.

## List of contents


| Title of content | | Page no. |
|---|---|---|
| الآيـــة | | I |
| Dedication | | II |
| Acknowledgment | | III |
| Abstract (English) | | IV |
| Abstract (Arabic) | | VI |
| List of contents | | VIII |
| List of tables | | XI |
| List of abbreviations | | XII |
| **Chapter one<br>Introduction and literature Review** | | |
| 1.1 | Introduction | 1 |
| 1.2 | Literature review | 2 |
| 1.2.1 | Normal hematopoiesis | 2 |
| 1.2.2 | Sites of hematopoiesis in pre-and post natal period | 4 |
| 1.3 | Anemia | 4 |
| 1.3.1 | Classification of anemia | 5 |
| 1.3.2 | Iron deficiency anemia | 6 |
| 1.4 | Iron metabolism | 6 |
| 1.4.1 | Iron absorption | 6 |
| 1.4.2 | Iron excretion | 7 |
| 1.4.3 | Iron storage | 7 |
| 1.4.4 | Plasma (transport) Iron | 8 |
| 1.5 | Causes of Iron deficiency anemia | 9 |
| 1.6 | Pathophysiology of Iron deficiency anemia | 10 |
| 1.7 | Clinical and laboratory feature | 11 |
| 1.8 | Menstrual pattern and iron store status in women of reproductive age | 12 |

| | | |
|---|---|---|
| 1.9 | Iron deficiency anemia during pregnancy | 14 |
| 1.9.1 | Iron need during pregnancy | 14 |
| 1.9.2 | Effect of maternal anemia and iron deficiency on mother and fetus | 15 |
| 1.9.3 | Prevention of iron deficiency during pregnancy | 17 |
| 1.9.4 | Treatment of iron deficiency anemia | 17 |
| 1.10 | Previous studies | 19 |
| 1.11 | Rationale | 20 |
| 1.12 | Objectives | 21 |
| 1.12.1 | General objective | 21 |
| 1.12.2 | Specific objectives | 21 |
| **Chapter two<br>Material and methods** | | |
| 2.1 | Study design | 22 |
| 2.2 | Study population and area | 22 |
| 2.3 | Inclusion criteria | 22 |
| 2.4 | Exclusion criteria | 22 |
| 2.5 | Sample size | 22 |
| 2.6 | Data collection | 23 |
| 2.7 | Methods | 23 |
| 2.7.1 | Sample collection | 23 |
| 2.7.2 | Haemo-analyzer procedure | 23 |
| 2.7.3 | Estimation of serum Iron | 24 |
| 2.7.3.1 | Procedure | 24 |
| 2.7.3.2 | Calculations | 24 |
| 2.7.4 | Estimation of total Iron binding capacity | 24 |
| 2.7.4.1 | Procedure | 24 |
| 2.7.5 | Normal ranges | 25 |

| | | |
|---|---|---|
| 2.8 | Ethical considerations | 25 |
| 2.9 | Data analysis | 25 |
| **Chapter three<br>Results** | | |
| 3.0 | Results | 27 |
| **Chapter four<br>Discussion, Conclusion and Recommendations** | | |
| 4.1 | Discussion | 34 |
| 4.2 | Conclusion | 35 |
| 4.3 | Recommendations | 35 |
| **References** | | 37 |
| **Appendices** | | 44 |

## List of tables


| No | Title | Page no. |
|---|---|---|
| 3.1 | Distribution of demographic variables | 28 |
| 3.2 | Frequency and severity of anemia | 29 |
| 3.3 | Association of anemic with demographic variables among study population. | 29 |
| 3.4 | Association of severity of anemia with demographic variables | 30 |
| 3.5 | Comparison of mean age, serum iron, TIBC and hematological parameters between anemic and normal participant | 31 |
| 3.6 | Comparison of mean serum iron and TIBC between pregnant and non-pregnant | 31 |
| 3.7 | Compression of serum iron and TIBC with demographic variables | 32 |

## List of abbreviations

| Abbreviation | Term |
|---|---|
| CBC | Complete blood count |
| CSFs | Colony stimulating factors |
| EDTA | Ethylene diamine tetra-acetic acid |
| Fe | Ferrous |
| FL | Femto liter |
| G.CSF | Granulocyte colony stimulating factor |
| G.I.T | Gastro intestinal tract |
| Hb | Hemoglobin |
| HSCs | Haematopoietic stem cell |
| IDA | Iron deficiency anemia |
| IL | Inter leukins |
| IUGR | Intra uterine growth retardation |
| Kg | Kilogram |
| MCH | Mean cell hemoglobin |
| MCHC | Mean cell hemoglobin concentration |
| MCV | Mean cell volume |
| Ng | Nano gram |
| Pg | Pico gram |
| PMB | Premature birth |
| PNH | Proximal nocturnal hemoglobinuria |
| Pt | Platelets |
| RBCs | Red blood cells |
| RPM | Round per minute |
| SCF | Stem cell factor |
| SI | Serum iron |
| TIBC | Total iron binding capacity |
| EPO | Erythropoietin |
| WBCs | White blood cells |

# Chapter one

# Introduction and literature review

# Chapter one

## Introduction and literature review

### 1.1 Introduction

Anemia is major health problem affecting both developing and developed countries with major consequence for human health as well a social and economic development .Anemia is defined as reduction in red blood volume or hemoglobin below the range of values occurring in healthy person. In Women of a childbearing age, the anemia prevalence is 30.2%; overall 468.4 million women of childbearing age are anemic (WHO, 2004).

According to Idris and Rehman (2005) reported that when iron deficiency is wide spread and sever, the prevalence of effect on the individuals resistance to infectious disease are significant. It has been reported to affect about 50_60% of young children and pregnant women and about 20_30 % of non-pregnant female in developing countries. The highest prevalence in Africa (47.5%) and in South-east Asia (35.7%). It is 17.8% in Americas (WHO, 2008).

Prevalence of anemia among women in reproductive age as percentage of women ages (15-40) In Sudan 31.5% as reported in 2011 (Stevens *et al*., 2013). It occurs in all stage of life but the more prevalent in pregnant women and young children. Pregnant women, during lactation and also during premenopausal period are most vulnerable for developing iron deficiency anemia (Mc Cann and Ames, 2007).

Iron deficiency range from depletion of tissue iron store to manifestation of full-blown hypo chromic micro cystic anemia. The manifestation of iron deficiency anemia are not only limited to reduced oxygen delivery to tissue but also include compromised cell proliferation, metabolism, biotransformation, immune mechanism, cardiac and cerebral function and overall growth, also iron deficiency anemia is reportedly the most common cause of anemia in general medical practice(Andrew *et al*.,2009). Thus iron deficiency is more than their

hematological disorder and affects all body organs. Anemia is some time treatable, but certain type of anemia may be long life. If the cause is dietary iron deficiency, treated by eating more iron rich food, or taking iron supplement, and alternatively, intravenous iron can be administered (Stephen *et al.,* 2009).

## 1.2 literature review

### 1.2.1Haematopoiesis

The formation of blood cellular components which derived from hematopoietic stem cell (Birbrair and Frenette, 2016).In a healthy adult person, approximately $10^{11}$_$10^{12}$ new blood cells are produced daily in order to maintain steady state level in the peripheral circulation (Parslow *et al.,* 2008).

Hematopoietic stem cell (HSCs) reside in the medulla of the bone marrow and have the unique ability to give rise to all of the different mature blood cell types and tissues .HSCs are self –renewing cell; when they proliferate, at least some their daughter cell remain as HSCs, soothe pool of stem cell is not depleted This phenomenon is called asymmetric division (Morrison and Kimble, 2006). The other daughters cell of HSCs (myeloid lymphoid progenitor cells) can follow any of the other differentiation pathway that lead to the production of one or more specific types of blood cell, but cannot renew themselves .The pool of progenitor is heterogeneous and can be divided into two group; long term self-renewing HSC and only transiently self-renewing HSC also called short terms (Morrison and Weissman, 2000).

All blood cells are divided into three lineages

-Erythroid cells are the oxygen carrying red blood cells. Both reticculocytes and erythrocytes are functional and are released into the blood.

-Lymphocytes are the cornerstone of the adaptive immune system .They are derived from common lymphoid progenitor. The lymphoid lineage is primarily composed of T-Cell and B-Cells. This is lymphopoiesis (Cumano and Godin, 2007).

-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.

-Megakaryocytopoiesis is hematopoiesis of megakaryocytes.

### 1.2.2 Sites of hematopoiesis in pre-and post natal period

In developing embryos, blood formation occurs in aggregates of blood cell in it's 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 (Birdbrain and Ferretted, 2016). However, maturation, activation and some proliferation of lymphoid cell occurs in the spleen, thymus, and lymph nodes. In children, hematopoiesis occurs in the marrow of the long bones such as femur and tibia. In adult, it occurs mainly in the pelvis, cranium, vertebrae, and sternum (Fernandez and De Alarcon, 2013). In some cases, the liver, thymus, and spleen may resume their hematopoietic function, if necessary. This known as extra medullary hematopoiesis. Andmay cause these organs to increase in size substantially (Georgiades *et al.,* 2002). Red and white blood cell production is regulated with great precision in healthy humans, and production of leukocytes is rapidly increased during infection (Tavian *et al.*, 2010*).*

### 1.2.2.1 Factors enhance hematopoiesis

The proliferation and self-renewal of these cells depend on growth factors. The main players in this process is called stem cell factor (SCFs) which bind to the c-kit receptor on the HSC. There are other important glycoprotein growth factor which regulates the proliferation and maturation, such as interleukins IL-2, IL-3, IL-6, and IL-7 ,colony stimulating factor (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) (Hauke*etal*.,2000). Erythropoietin is required for myeloid progenitor cell to become an erythrocyte. In the adult, EPO is primarily produced in kidney as a response to hypoxic induction of the epo-gene. EPO then binds to erythropoietin receptors (EPOR), initiating signaling that stimulates growth, inhibits apoptosis, and induces differentiation of erythroid progenitors to increase red cell mass (Lacombe and mayeux, 2000). Thrombopoietin is produced in the liver by both parenchymal and sinusoidal endothelial cells, as well as in the kidney by proximal convoluted cells. In the liver, it`s production is augmented by (IL-6). Thrombopoietin regulates the differentiation of megakaryocytes and Platelets (Kaushan-sky, 2006).

## 1.3 Anemia

Anemia defines as a decrease in both red cell mass and hemoglobin concentration or decrease in hematocrite when compared with normal group. Anemia is functionally defined as decrease in the competence of blood to carry oxygen to tissue thereby causing tissue hypoxia (Milman *et al.,* 2005). The symptoms of anemia depend upon the degree of reduction in the oxygen-carrying capacity of the blood, change in the total blood volume ,the rate at which these change occurs ,the degree of severity of the under lying disease contributing to anemia , and the power of the cardiovascular and hematopoietic systems to recuperate and compensate (Maakaron and Joseph, 2016).

### 1.3.1 Classification of Anemias

Anemia can be caused by the defective production of red cells or an increased rate of loss of cells, either by bleeding or premature destruction (haemolysis). The causes of defective production of red cells include:

- Deficiency of iron, vitamin B12 or folate.

- Anemia of chronic disorders.

- Reduced erythropoietin production –chronic kidney disease.

- Primary disease of bone marrow (Mitchel *et al.*, 2007).

Hemolytic anemias –causes are

- Genetic-including membrane defects, hemoglobindisorders and enzyme deficiencies.
- Acquired–including autoimmune and non immunodisorders (Weatherall & ChrisHatton, 2003).

The main causes of anemia can be usefully classified according to the associated red cell change:

- Hypo chromic, microcytic –including iron deficiency, thalassaemia.
- Normochromic, macrocytic vitamin B12 and folat deficiency, alcohol.
- Poly chromatophilic, macrocytic –hemolysis.
- Normochromic, normocytic –chronic disorders, renal failure, disease of the bone marrow.
- Leucoerythroblastic myelofibrosis, leukaemia, metastatic carcinoma. (Weather all and ChrisHatton, 2003).

### 1.3.2 Iron deficiency anemia

Iron deficiency anemia is the most common nutritional deficiency in the world. Occurs when a level of red blood cells (RBCs) in the blood that is lower than normal. It cause reduce work capacity in adult and impact motor and mental development in children and adolescents (Halterman *et al.*, 2001). There are some evidence that iron deficiency without anemia affects cognition in adolescent girls and causes fatigue in adult women, and may affect visual and auditory functioning and is weakly associated with poor cognitive development in children (Verdon *et al.*, 2003).

### 1.4Iron metabolism

Iron metabolism is unusual in that it is controlled by absorption rather than excretion. Iron is only lost thruoght blood loss or loss of cell as they slough. Menstruating women lose from 0.2 to 2.5 percent more days. An average (60-kg) woman might lose an extra 10 mg of iron per menstruation cycle, but the loss could be more than 42 mg cycle depending on how heavily she menstruates. Pregnancy take about 700mg of iron, and whole blood donation of 500cc contain 250mg of iron (Wintrob and Lee, 2000).

### 1.4.1Iron absorption:

It’s occurs mostly in jejunum and only 5to10 %of dietary intake in person in homeostasis .In state of overload, absorption decreases (Geissler and Powers, 2005).

Absorption can increase three –to fivefold in state of depletion. Dietary iron is available in two forms: hemeiron, which is found in meat; and non heme iron, which found in plant and dairy foods. Absorption of heme iron is minimally affected by dietary factors, whereas non heme iron make up of bulk of consumed iron. The bioavailability of non heme iron requires acid digestion and varies by an order of magnitude depending on the concentration of enhancer (e.g., ascorbate, meat) and inhibitors (e.g.: calcium, fiber ,tea coffee, wine) found in the diet(Wintrob and Lee,2000).

### 1.4.2 Excretion

Iron losses occurred in normal individuals mainly in faces (0.6mg/day), bile and desquamated mucosal cells, and in minute of blood.

Other ways of iron loss include desquamated skin and sweat (0.2-0.3mg/day).urine (<0.1mg/day) (Semba and Ramakrishnan, 2008). Men require about 1mg(range 0.5-2.0 mg/day) iron daily each day, and this sufficient to balance the daily losses from desquamation of epithelia (Gibney *et al*.,2007).

### 1.4.3 Iron storage

The amount of storage iron has been estimated to be and about 1000-2000 mg in the healthy adult male, and less in the female .storage iron occur in two forms – ferritin and hemosiderin .Ferritin is normally predominant. In normal person storage iron is divided about equally between the reticuloendothelial cell (mainly in spleen, liver and bone marrow), hepatic parenchyma cell and skeletal muscle (Gibney *et al*., 2007).

Hemosiderin, the main storage form in reticuloendothelial cells, iss more stable and less readily mobilized for hemoglobin formation than ferritin, which predominantes in hepatocytes. In states of iron overload, hemosidern increases to a great degree than ferritin and became the dominant storage form (mehata and Hoffbrand, 2000).

### 1.4.4 Plasma (transport) Iron

Between 3 and 4 mg iron present in the plasma, where is bound to specific protein, transferrin , a B-globulin of molecular weight 88000 that is synthesized in the liver each molecule of tranferrin binds one or two atoms of ferric iron s(Shayeghi *et al.*,2005).

The function of transferrin is the transport of iron. It is the means by which iron absorbed from the alimentary tract is transported to the tissue stores, from the tissue stores to bone marrow erythroblasts, and from one storage site to another. When transferrin reaches the storage sites or the bone marrow, it attached to specific receptor on the cell and liberates its ferric iron, which passes into the cell to be stored or utilized. Total amount of transferrin in the plasma about 8g in the extracellular fluid. The level of serum iron in normal subjects average about 20 mmol (men>women) (Hoffbrand and Pettit, 2000).

Transferrin is a glycoprotein present in the serum in concentration, which enable it to be combined with 44-80mmol of iron per liter. This value is known as total iron binding capacity of the serum (Pennon *et al*., 2000)

Serum ferritin concentration in adult`s range between 15-300mg /l and the mean level in men and women are 123mg/l and 56mg/l. In iron deficiency, concentration is less than 12mg/l. In iron over load, levels are very high. Serum ferritin increased in: infections, inflammations, malignancy and liver disease (Hoff brand and Pettit, 2000)

## 1.5 Causes of iron deficiency

### 1.5.1 Increased physiological requirement

- Growth: anemia is more common in children between age of 6 month and 2 years, and from 11 to 16 years due to spurts of growth during these periods.

- Menstruation: anemia common in adult menstruating females.

- Pregnancy: during pregnancy anemia is almost universal (Hoffbrand and Pettit, 2000).

### 1.5.2 Pathological blood loss

- Menorrhagia-Antepartum and post-partum bleeding.

**- Gastrointestinal tract**

Bleeding piles
Drugs: aspirin, indomethacin, Butazolidin, and Corticosteroids, Pepticulcer.

Intestinal infection and infection: Ankylostoma, whipworm, chronic colitis

Due to amoebic or baillary infection and giardiasis,

Miscellaneous: Cirrhosis of liver, hiatus, hernia, T.B bowels (Bermejo and Garcia, 2006).

- Malabsorption; coeliac disease, postgastrectomy and atrophic gastritis.
- Urinary tract: recurrent hematouria and hemoglobinuria
- Regular blood donation
- Rescurrent hemoptysis
- Pulmonary hemosiderosis (Coad and conlon, 2011).

### 1.5.3 Nutritional defect

- Low iron intake.
- Inhibitor in diet

### 1.5.4 Excess iron loss

Exfoliative dermatitis, PNH, GI infection, and Intravenous hemolysis (Brabin *et al.,* 2001).

## 1.6 Pathophysiology of iron deficiency anemia

Iron deficiency develops in sequential stages over a period of negative balance (loss exceeds absorption).

Since Fe is absorbed with difficulty, most people barely meet their daily requirements added losses due to menstruation (mean 0.5 mg\day ), pregnancy (0.5 to0.8 mg\day), lactation (0.4mg\day)and blood loss due to disease or accident readily lead to Fe deficiency .Fe depletion results in sequential changes or stages (Smith, 2010).

**Stage 1 (Depletion of iron stores):** Fe loss exceeds intake; with this negative balance, storage Fe (represented by bone marrow fee content) is progressively depleted. Although the Hb and serum Fe remain normal, the serum ferritin concentration fall to<20 ng/ml. As storage Fe decrease, there is compensatory increase in absorption of dietary Fe and in the concentration of transferrin (represented by a rise in Fe binding capacity (Crichton, 2009).

**Stage 2 (impaired erythropoiesis):** Exhausted Fe cannot meet the need of erythroid marrow ,while the plasma –trasferrin level increases ,the serum Fe concentration declines ,leading to progressive decrease in Fe transferrin saturation to<16%,erythropoises is impaired. Serum ferritin receptor concentration raises (>8.5mg/l) (Harvey, 2000).

**Stage 3(Anemic stage):** the anemia with normal appearing RBCs and the indices is defined (Weiss, 2010).

**Stage 4:** Microcytosis and then hypochromia are present. The most significant finding is the classic microcytic hypochromic anemia (Anderson *et al.*, 2000).

**Stage 5:** Fe deficiency affects tissue, resulting in symptoms and signs.

### 1.7 Clinical and laboratory features of iron deficiency anemia

In early stage, there is no clinical manifestation .But with complete depletion of iron stores, anemia develops and clinical symptoms appear. Symptoms such as weakness and lethargy are considered to be related to hypoxia cause by the decrease in hemoglobin. Variety of other abnormalities may occur from an absence of tissue iron in iron-containing enzymes .These include koilonychias (concavity of nail) glossitis, pharyngeal webs, muscle dysfunction, impaired thermo genesis and gastritis (Elnicki *et al*., 2001).

The most common dysphagia described in patients with IDA includes ice-eating (phagophagia), dirt eating (geophagia) and starch eating (amylophagia) (Cook, 2005). The blood picture is well –developed iron deficiency is microcytic (MCV55 to 74fl), hypochromic (MCHC 22 to31g/dl, MCH 14 to 26pg).When the anemia mild, the morphologic aspects of the red cells are little affected. Microcytosis and anisocytosis are usually the first morphologic signs to develop even before anemia develops (Tefferi, 2003).

The blood film demonstrates progressive poikilocytosis. The most frequent poikilocytosis are target cell and elliptocytosis. Nucleated red cell may be seen if hemorrhage has occurred. Both the relative and absolute number of reticulocyte may normal or slightly increased (Cook, 2005).

WBC is usually normal but some time eosinophilia may be present .While Platelets may be normal, increased or decreased. Thrombocytopenia may occur in sever or long-standing anemia especially if associated with folate deficiency .It has proposed that thrombocytosis related to iron deficiency caused by chronic blood loss (Stephen, 2009).

**Iron studies** : the serum iron is decreased and usually less than 30mg/dl ,TIBC is increased with less than 15% transferring saturation .Serum ferritin level are decreased in all stages of IDA and may be the first indication of developing iron deficiency (Gupta and Kalia,2004).

Bone marrow characterized by decrease myloid: erythroid ratio, moderate increased of cellularity and mild to moderate erythroid hyperplasia. In erythroid series there is poorly hemoglobin zed normoblasts with scanty ragged cytoplasm and erythroid nuclear abnormalities (nuclear fragment, multinuclearity). The stains for iron shows absence of hemosiderin in the macrophage and sidroblast are markedly reduced or absent (Loannou, 2002).

### 1.8 Menstrual pattern and iron store status in women of reproductive age

Duration of menstruation is usually around 3-5 day and considered abnormal if lasts for more than 7 day. There is blood loss during menstruation .Longer the duration of menstruation is more blood lost associated with iron loss and caused iron deficiency anemia. The amount of blood loss indirectly known by the number of sanitary napkins is used every day. It is classified as abnormal if the sanitary napkins are changed every 3 hours or more than 6 time daily (Brawan *et al* ., 2011).

Iron is not actively excreted our body through urine or intestine, iron loss together with cell from skin ,mucous membrane ,urinary tract and respiratory tract. The estimated amount of iron loss was 14 microgram/kg BW/day .The range of individual variation is estimated to be +or-15%. Iron loss during sweating is considered, especially in hot and moist climate. However, based on the newest research that applied extensive protection to prevent interference of iron contamination from the skin during the collection period of total body sweats, iron loss through sweating can be abandoned. (Semba and Ramakrishnan, 2008).

Iron loss during menstruation is slightly constant in every woman but has significance variable between one women to another. The main part of this variation is genetic problem which is controlled by fibrinolytic activator within mucous membrane of uterus across population in geographically far separated population. The amount of menstrual iron loss during the whole menstrual cycle for 28 days is around 0.56 mg per day. In addition to basal iron loss (0.8mg/day) and its variation, distribution of total iron demand in women can be counted as total amount between distribution of menstrual and basal iron loss (Raharjo, 2003).

The mean daily iron requirement is 1.36 mg, for10% of women the requirement increased to 2.27mg, and 5% of women requirement is 2.84mg (Djazayery, 2001). In10%of young adult women (during growth period ), daily total iron demand could reach 2.65mg and in 5% young adult women could reach3.2 .Due to the skewness of normal distribution of menstrual iron loss ,it could be a huge nutritional problem because the estimation of individual iron loss could not be fully trusted. It meant that women with physiological iron loss but sever from could not be identified and managed with iron supplementation (Janze *et al*., 2013).

## 1.9 Iron deficiency anemia during pregnancy

A high proportion of women in both developing and industrialized countries became anemic during pregnancy .Estimates from the World Health Organization reported that from 35% to 75% (56% on average) of pregnant women in developing countries ,and 18 % of women from industrialized countries .However many of this women are anemic were already anemic at the time of conception , with an estimated prevalence of anemia of 43% in non-pregnant women in developing countries and 12% in women in wealthier region (WHO,2005).

The prevalence of iron deficiency is far greater than the prevalence of anemia and iron deficiency (low serum ferritin, and sparse or absent stainable iron in bone marrow) often develops during the later stage of pregnancy even in women who enter pregnancy with relatively adequate iron store (Schorr and Hediger, 1994).

### 1.9.1 Iron needs during pregnancy

During pregnancy, the average total iron requirement has been estimated to approximately 1200mg for a weight of 55kg in pregnant women. The iron is used mainly for the increase in maternal erythrocyte mass (450 mg) ,placenta (90mg), fetus(250-300mg) general loss (200-250mg) and blood loss at delivery corresponding to 150mg iron(300-500ml blood loss). Around 40 % of women begin their pregnancy with low or absent iron store (serum ferritin<30 micro gram/l) an up to 90% insufficient to meet the increase iron need during pregnancy and post-partum .Iron absorption requirement in the first trimester are around 0.8 mg/day, raising to7.5mg/day at the third trimester(Lee and Okman2011).

### 1.9.2Effect of maternal anemia and iron deficiency on mother and fetus

#### 1.9.2.1Maternal risks

Iron deficiency influence whole series of body functions , such as physical and mental performance , enzymatic functions (e.g., those of respiratory chain) , thermoregulation ,muscular functions ,the immune response and neurological function (Martius .2009). Only a few of these potential effects have been specifically investigated in iron deficiency anemia. In general IDA lead to numerous symptoms such as fatigue, a reduction in physical performance and fitness for work, increased cardiovascular stress, reduce thermoregulation and an increased predisposition to infections. Maternal thyroid function and synthesis of thyroxin is closely linked to maternal iron status. (Kazi *et al.,* 2010).

In the gravida, the tolurace for preipartum blood loss is greatly reduced. Maternal mortality increases depending on the severity of the IDA. Causes include an increased rate of cardiovascular failure, a high risk of hemorrhagic shock and higher rates of infection during puerperium and impaired wound healing (Milman *et al.,* 2005).

Maternal morbidity may also be associated with socio-economic status, the level of medical care, nutritional status. General problem in interpreting the available studies is that the maternal and fetal outcome have been investigated in relation to the severity of anemia, but not in relation to the duration or initial onset of anemia (Balarajan *et al.,*2013).

### 1.9.2.2Fetal risks

Maternal Hb levels below 9.0 g/dl increase the risk of premature births (PMB), intrauterine growth retardation (IUGR) and intrauterine fetal death (IUFD). The association between maternal Hb and birth weight follows a U-shaped curve .Hb level of more than 11.0 g/dl and less than 9.0g/dl are associated with tow-to three –times greater risk of a light –for-dates neonate .Hb level of more than 12.0 g/dl at the end of second trimester are associated with an increased risk of preeclampsia and IUGR, probably due to a lake of plasma volume expansion. The `ideal` Hb range with respect to the prevention of prematurity and IUGR babies appears to lie between 9.5 and 11.5 g/dl (Murray *et al.*, 2009).

There is increasing evidence of an association between the timing and duration of iron deficiency and anemia, and of pathological feto- placental changes. The risk of a premature birth is increased if there is iron deficiency during early pregnancy. However, it is not clear whether this primarily the result of a lake of oxygen supply, or more the consequence of iron not being released or utilized (Schorr and Hediger, 1994).

Serum ferritin levels are too low, indicating depleted iron stores; appear to have association with growth retardation, while the correlation with high serum ferritin levels is less evident. Even with adequate or balanced diet, iron supplementation, especially during the third trimester reduces the risk for a small gestational age baby (Haider *et al.*, 2013).

### 1.9.3Prevention of iron deficiency during pregnancy:

Most guidelines recommend an increase in iron consumption by about 15 mg/day (to about 30mg/day), an amount readily met by most prenatal vitamin formulation. This is adequate supplementation for non-anemic and noniron deficient women. In a 2012 systematic review, daily iron supplementation reduce the risk of maternal anemia at term by 70% and iron deficiency at term by 57% .According to WHO guidelines, daily oral iron and folic acid supplementation is recommended as part of the antenatal care to reduce the risk of low birth weight, maternal anemia and iron deficiency (WHO, 2012). Intermittent iron supplementation for preventing anemia at term and is better tolerated. Women with IDA (first or third trimester Hb <11 g/dl or second trimester Hb <10.4 g/dl and low serum ferritin) should be received an additional iron supplement of 30-120mg/day until the anemia is corrected (Milman *et al.*, 2005).

### 1.9.4Treatment of iron deficiency anemia

#### 1.9.4.1 Oral iron

Replacement therapy with gradual replenishment of iron stores and restoration of Hb is the first –line treatment for most patients with IDA (Cook, 2005).

**Dosage:** The dose of oral iron for IDA should be from 30 to 80 mg of elemental iron daily, given for 3 to 6 months and for longer if the cause of iron deficiency is ongoing. Depending on the cause of IDA, Hb concentration should rise by 0.5 to 1g/dl (5-10g/l) per week. Oral ferrous salts are the treatments of choice as ferric salt are less well absorbed (Fogelholm *et al.*, 1994).

### 1.9.4.2 An intravenous iron indications

- When oral iron is not tolerated or ineffective in raising or in maintaining Hb concentration.

- When Hb is <10g/dl (depends on clinical setting).

- When anemia is symptomatic.

- Chronic inflammatory disease, chronic renal failure, chemotherapy-induced anemia (Barton *et al.,* 2000).

### 1.9.4.3 Selection and dosing of intravenous iron

The formulations of intravenous iron now available in Europe are sodium ferric gluconate (Ferrlecit), iron (3)-hydroxide dextran complex (Cosmofer), ferric carboxymaltose (Ferinject) (Barton *et al.,* 2000).

The Ganzoni formula calculates the total iron deficit requiring intravenous replacement as: body weight (kg)*{target Hb-actual Hb} [g/l]*0.24+iron store [mg], where the iron stores for patient >35kg are assumed to be 500mg. In contrast, the Simplified Method, derived initially from trial using carboxy-maltose in patients with inflammatory bowel disease allows calculation of the dose of iron needed from the patients Hb concentration and weight (Aspuru *et al.,* 2011).

The response to intravenous iron should be determined by monitoring the Hb concentration, transferring saturation, and /or ferritin levels at about 6 weeks after infusion (Barton *et al*., 2000).

## 1.10 Previous studies

Ashish bancal and his team in 2016 conclude that in study performed in India among female at reproductive age, the largest number (43%) suffering from moderate anemia ,72% were having microcytic hypochromic anemia, mean serum ferritin (10.8U/L) much below the normal values, which linked with low iron intake.

There was significant correlation between iron deficiency anemia and iron deficiency with inadequate meat intake and impaired exercise capacity, reported by Fatin and her coworkers in 2011 in case control study in Saudi Arabia (fatin *et al*., 2011).

Other study in Khartoum state, Sudan 2011 among women at reproductive age done by Abdella and his college ,was releaved that means of hemoglobin ,hemtocrit ,and RBCs count were statistically significance lower than means of control group(*P* value <0.05), and the frequency of iron deficiency anemia was (24.8%) (Abdallh, 2011).

Prevalence of anemia among women of reproductive age (% of women ages 15-49) in Sudan was 31.5% at 2011. Its highest value over the past 16 year was 40.80 in 1995, while its lowest value was 31.5% in 2011(Stevens *et al.*, 2013).

## 1.11 Rational

Iron deficiency anemia is dimorphic anemia due to deficiency of iron and folate, which is highly prevalent in community. However, large number of cases remains undiagnosed. It important to confirm iron deficiency using Hb level, Serum iron and total iron capacity (Mateo's *et al.*, 2009).

A poor in women of childbearing age the most common cause of iron deficiency anemia as well as loss of iron in the blood due to heavy menstruation or pregnancy diet or certain intestinal disease that affect body iron absorption can also cause iron deficiency anemia (Godder *et al.*,2011).

There was no available information regarding iron deficiency anemia among Sudanese female during childbearing age that depend on traditional food as main source of dietary iron.

## 1.12 Objectives

### 1.12.1 General objective

To detect the frequency of iron deficiency anemia among females in reproductive period in Khartoum State, Sudan.

### 1.12.2 Specific objectives

1. To estimate iron level and total iron binding capacity in Sudanese females at reproductive age.
2. To correlate the relationship between severity of iron deficiency anemia with pregnancy, food intake and menstrual pattern.
3. To correlate anemia and body iron status to women demographic data.

# Chapter two

# Materials and methods

# Chapter two

# Materials and Methods

## 2.1 Study design and duration

This was a descriptive cross-sectional study conducted in the period from January-April 2017 at Khartoum State.

## 2.2 Study population and area

The study was carried out in women at reproductive age group (15-47) years, who attended to the outpatient's clinic at Saad Abo Elela Hospital, Khartoum State.

## 2.3 Inclusion criteria

This study was designed to include female only between ages of (15-47) years.

## 2.4 Exclusion criteria

Those reported with chronic diseases (such as diabetes mellitus, hypertension) or hereditary anemias, breast feeding and women who takes contraceptive pills and previous blood transfusion in the last 3month were excluded.

## 2.5 Sample size

Total of 100 female between ages of 15 to 47 years were selected from population of Khartoum State.

## 2.6 Data collection:

Data for nutrition status and other parameter were collected with informed consent agreement; each woman interviewed to complete a structured questionnaire, which included personal information on age, dietary information, medical history and menstrual history and hematological data.

## 2.7 Methods:

### 2.7.1 Sample collection

Venous blood sample(5ml) was collected using sterile disposable plastic syringe after cleaning the veinopuncture area with 70% ethanol ,the sample was divided into two part,(2.5ml) was added into ethylene diamine tetra-acetic acid (EDTA) used to analyze complete blood count included hemoglobin ,mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC),mean cell volume(MCV), haematocrit, red blood cell count (RBC) and platelets was analyzed by using blood counter (SYSMEX Kx21).

The other (2.5ml) of blood was drawn into plain tube. Serum separated by centrifuging blood for 10 minute at 3000RPM and then labeled with data name and identification number of volunteer participating in this study. And stored frozen at 4c for 3 days to biochemical analysis of serum iron and total iron binding capacity on spectrophotometer at absorbance 540 nm.(Iron deficiency is defined as hemoglobin less than 11mg/dl).

### 2.7.2 Haemo-analyzer procedure

Sample was collected and drawing into a test tube containing an anticoagulant (EDTA, sometime citrate) to stop clotting. The blood will mixed and placed on a rack in analyzer. Then this instrument flow the cells, photometer and apertures that analyze different element of blood.

### 2.7.3 Estimation of serum iron concentration

#### 2.7.3.1 Procedure

0.5 ml serum (free of haemolysis), 0.5 ml working iron standard and 0.5ml iron free water (as blank) were placed respectively, in each of three 1.5ml polypropylene micro –centerifuge tubes with lid and then 0.5 ml protein precipitant was added to each and replaced the lid. the content mixed vigorously and allowing to stand for 5 min. Centrifuge the tube containing the serum at 13000 g for 4 min to obtain the clear supernatant .To 0.5 ml of this supernatant and to 0.5 ml of each other mixture added 0.5ml chromogen solution with thorough mixing .After standing for 10 min, measured the absorbance in spectrophotometer against water at 560nm.

If the micro-centrifuge is not available, use double volume of serum and reagents in a 3ml plastic tube with lid and centrifuge at 1500 g for 15 min in bench centrifuge.

#### 2.7.3.2 Calculation

$$\boldsymbol{serum\ iron(mmol)} = \frac{(\text{Absorbance of test} - \text{Absorbance of blank}) \times 8}{(\text{Absorbance of standard} - \text{Absorbance of blank})}$$

### 2.7.4 Estimation of total iron binding capacity

#### 2.7.4.1 Procedure

0.5 ml serum was placed in a 1.5ml polypropylene micro centrifuge tube and added 0.5ml saturating iron solution. Mix carefully by hand leave at room temperature for 15min. a plastic tube was used to add 100mg light magnesium carbonate and cap the tube shaking vigorously and allowed to stand for 30min with occasional mixing .Centrifuge at 13000 g for 4min in a micro centrifuge . If the supernatant contains traces of magnesium carbonate, remove the supernatant and recenterifuge. Carefully removed 0.5ml supernatant and was treated as serum for iron estimation .Multiply the final result by 2.

### 2.7.5 Normal range

- Serum iron: 60-170 micro gram/l

- Total iron binding capacity: 250-450 micro gram/l.

### 2.8 Ethical considerations

Participants were informed verbally with simple language about the research, its benefits and method of sample collection, then their approval was taken.

### 2.9 Data analysis

The data were analyzed by using Statistical Package for Social Sciences (SPSS version 11.5) for windows version 7 using T- dependent test for testing significance difference and chi-squire test used to assess the association of anemia with demographic data of study group. One-way ANOVA test was used to show the comparison of iron status with demographic data of women. P-*Value* $\leq 0.05$ was considered statistically significance.

# Chapter three

# Results

# Chapter three
## 3. Results

A cross sectional was study carried out at Saad Abo Al-ela hospital in Khartoum State.100 sample was collected from healthy women from January to April 2017.Demographic data of respondent was collected. Women enrolled in this study with mean age 25.9±6.4 and age classified into three age group ,the more frequent one was 15-25 years (56/100,56%) followed by age group 26-36(35/100,35%).

While the last one age 37-47 years was lowest frequency (9/100,9%).Unmarried women were highest frequency compared with married one(53%,47%) respectively and pregnant women represented 30% compared with 70% non-pregnant .About 8% experiencing abnormal menstrual bleeding , 33% of women with low iron intake,41% moderate and 26%high iron intake. Regard to the educational level, 6% of women had illiterate, 31% had higher education level (Table 3.1).

Most of women were having moderate anemia (55.6%), (14.8%) with mild anemia and (29.6%) have severe anemia. The prevalence of anemia was 27% (Table 3.2).

An association of anemic status with demographic variables was not statistically significance (Table 3.3).The results showed no statistically significance association between the severity of anemia and demographic variables (Table 3.4). By using independent T-test the comparison of mean age, SI, TIBC, and hematological parameter between anemic and non-anemic were done, we found that mean age ,SI and TIBC in normal ($26.3 \pm 6.6, 71.8 \pm 7.8, 225.9 \pm 57.6$) respectively, and in anemic(24.7±5.6, 55.7±6.6, 328±82.7) respectively. All values showed statistically significance difference (*P-value* <0.05), except in mean of age (*P-value* =0.256) (Table 3.5). The comparison in hematological parameters between pregnant and non-pregnant also showed no statistically significance difference in mean of serum iron ($p$=0.27) and TIBC ($p$=0.39). But in mean of MCH, MCHC, .and RBCs show significant difference (Table 3.6).

Fainally, we expressed the comparison of serum iron and TIBC with demographic variables by using One-way ANOVA test, were found significance difference in serum iron and TIBC between different group of iron intake (*P-value*= 0.01) (Table3.7).

**Table (3- 1) Distribution of demographic variables**

| **Variables** | **Frequency** | **Percent** |
|---|---|---|
| **Age Group** | | |
| 15 to 25 years | 56 | 56 |
| 26 to 36 years | 35 | 35 |
| 37 to 47 years | 9 | 9 |
| **Marital status** | | |
| Single | 53 | 53 |
| Married | 47 | 47 |
| **Pregnancy** | | |
| Not pregnant | 70 | 70 |
| Pregnant | 30 | 30 |
| **Trimester** | | |
| **First** | 11 | 36.7 |
| **Second** | 12 | 40 |
| **Third** | 7 | 23.3 |
| **Menstrual days** | | |
| 3 | 10 | 14.3 |
| 4 | 4 | 5.7 |
| 5 | 16 | 22.9 |
| 6 | 18 | 25.7 |
| **7** | 16 | 22.9 |
| 8 | 6 | 8.6 |
| **Iron intake** | | |
| Low | 33 | 33 |
| Moderate | 41 | 41 |
| High | 26 | 26 |
| **Education** | | |
| Illiterate | 6 | 6 |
| Primary | 31 | 31 |
| Secondary | 11 | 11 |
| University | 52 | 52 |

**Table (3-2) Frequency and of severity of anemia**

| Anemia | Frequency | Percent |
|---|---|---|
| Normal | 73 | 73 |
| Anemic | 27 | 27 |
| **Severity** | | |
| Mild | 4 | 14.8 |
| Moderate | 15 | 55.6 |
| Severe | 8 | 29.6 |
| Total | 27 | 100 |

**Table (3-3) Association of anemic with demographic among study population**

| **Variables** | **Anemia** | | | ***P.value*** |
|---|---|---|---|---|
| **Age group** | Normal | Anemic | Total | |
| 15-25 years | 39 | 17 | 56 | 0.424 |
| 26 to 36 years | 26 | 9 | 35 | |
| 37 to 47 years | 8 | 1 | 9 | |
| **Marital status** | | | | |
| Single | 37 | 16 | 53 | 0.446 |
| Married | 36 | 11 | 47 | |
| **Menstrual day** | | | | |
| 3 | 8 | 2 | 10 | 0.263 |
| 4 | 2 | 2 | 4 | |
| 5 | 12 | 4 | 16 | |
| 6 | 15 | 3 | 18 | |
| **7** | 11 | 5 | 16 | |
| 8 | 2 | 4 | 6 | |
| **Pregnancy** | | | | |
| Nonpregnant | 50 | 20 | 70 | 0.589 |
| Pregnant | 23 | 7 | 30 | |
| **Trimester** | | | | |
| First | 9 | 3 | 12 | 0.864 |
| Second | 9 | 2 | 11 | |
| Third | 5 | 2 | 7 | |
| **Iron intake** | | | | |
| Low | 20 | 13 | 33 | 0.099 |
| Moderate | 34 | 7 | 41 | |
| High | 19 | 7 | 26 | |
| **Education** | | | | |
| Illiterate | 4 | 2 | 6 | 0.648 |
| Primary | 25 | 6 | 31 | |
| Secondary | 7 | 4 | 11 | |
| University | 37 | 15 | 52 | |

**Table (3-4) Association of severity of anemia with demographic variables**

| **Variables** | **Severity of anemia** | | | | ***P.value*** |
|---|---|---|---|---|---|
| **Age Group** | Mild | Moderate | Severe | Total | |
| 15 to 25 years | 2 | 11 | 4 | 17 | 0.468 |
| 26 to 36 years | 2 | 3 | 4 | 9 | |
| 37 to 47 years | 0 | 1 | 0 | 1 | |
| **Marital status** | | | | | |
| Single | 3 | 10 | 3 | 16 | 0.314 |
| Married | 1 | 5 | 5 | 11 | |
| **Menstrual day** | | | | | |
| 3 | 0 | 1 | 1 | 2 | 0.283 |
| 4 | 0 | 1 | 1 | 2 | |
| 5 | 0 | 3 | 1 | 4 | |
| 6 | 1 | 1 | 1 | 3 | |
| 7 | 3 | 2 | 0 | 5 | |
| 8 | 0 | 3 | 1 | 4 | |
| **Pregnancy** | | | | | |
| Non pregnant | 4 | 11 | 5 | 20 | 0.232 |
| Pregnant | 0 | 4 | 3 | 7 | |
| **Trimester** | | | | | |
| 1 | 0 | 1 | 1 | 2 | 0.227 |
| 2 | 0 | 1 | 2 | 3 | |
| 3 | 0 | 2 | 0 | 2 | |
| **Iron intake** | | | | | |
| Low | 3 | 8 | 2 | 13 | 0.32 |
| Moderate | 1 | 3 | 3 | 7 | |
| High | 0 | 4 | 3 | 7 | |
| **Education** | | | | | |
| Illiterate | 0 | 0 | 2 | 2 | 0.145 |
| Primary | 1 | 5 | 0 | 6 | |
| Secondary | 2 | 8 | 5 | 15 | |
| University | 1 | 2 | 1 | 4 | |

**Table (3-5) shows comparison in mean age, serum iron, TIBC and hematological parameters between anemic and normal participant**

| Varible | Normal mean± STD | Anemic(mean± STD) | *P. value* |
|---|---|---|---|
| **Age** | 26.3±6.6 | 24.7±5.6 | 0.256 |
| **Serum iron** | 71.8±7.8 | 55.7±3.8 | 0.000 |
| **TIBC** | 225.9±57.6 | 328±82.7 | 0.000 |
| | | | |
| **MCV** | 85.2±5.1 | 73.6±6.3 | 0.000 |
| | | | |
| **MCH** | 27.9±2.7 | 23.4±3.4 | 0.000 |
| | | | |
| **MCHC** | 32.8±2.0 | 29.8±2.2 | 0.000 |
| | | | |
| **Hb** | 11.9±1.2 | 9.4±1.6 | 0.000 |
| | | | |
| **RBCs** | 4.3±0.38 | 4.0±0.50 | 0.002 |
| | | | |
| **WBCs** | 6.7±2.2 | 6.3±2.1 | 0.495 |
| **PLt** | 310±100 | 382±129 | 0.004 |

**Table (3-6) Comparison of mean serum iron and TIBC between pregnant and non-pregnant**

| | Pregnant(Mean±STD) | Non pregnant(mean± STD) | *P. value* |
|---|---|---|---|
| **Serum iron** | 69.2 ±10.2 | 66.8 ±9.9 | 0.278 |
| **TIBC** | 244.4±62.1 | 257.5±85.7 | 0.393s |
| **MCV** | 82.4±5.8 | 81.9±8.1 | 0.764 |
| **MCH** | 28.2±2.5 | 26.0±3.8 | 0.001 |
| **MCHC** | 33.3±1.7 | 31.4±2.5 | 0.000 |
| **Hb** | 11.7±1.5 | 11.0±1.8 | 0.104 |
| **RBCs** | 4.0±0.47 | 4.2±47 | 0.037 |
| **WBCs** | 6.7±1.9 | 6.5±2.3 | 0.629 |
| **PLt** | 290.6±102 | 346.3±113 | 0.023 |

**Table (3-7) shows caparison of serum iron and TIBC with demographic variables**

| **1** | **comparison in serum iron and TIBC in different age groups** | | | | | |
|---|---|---|---|---|---|---|
| | | Sum of Squares | df | Mean Square | F | Sig. |
| serum iron | Between Groups | 28.658 | 2 | 14.329 | 0.14 | 0.87 |
| | Within Groups | 9934.252 | 97 | 102.415 | | |
| TIBC | Between Groups | 3835.189 | 2 | 1917.595 | 0.3 | 0.741 |
| | Within Groups | 619810.8 | 97 | 6389.802 | | |
| **2** | **comparison in serum iron and TIBC in different education levels** | | | | | |
| | | Sum of Squares | df | Mean Square | F | Sig. |
| serum iron | Between Groups | 235.165 | 3 | 78.388 | 0.774 | 0.512 |
| | Within Groups | 9727.745 | 96 | 101.331 | | |
| TIBC | Between Groups | 39133.37 | 3 | 13044.46 | 2.142 | 0.1 |
| | Within Groups | 584512.6 | 96 | 6088.673 | | |
| **3** | **comparison in serum iron and TIBC in different trimesters** | | | | | |
| | | Sum of Squares | df | Mean Square | F | Sig. |
| serum iron | Between Groups | 438.212 | 2 | 219.106 | 2.244 | 0.125 |
| | Within Groups | 2636.588 | 27 | 97.651 | | |
| TIBC | Between Groups | 4425.304 | 2 | 2212.652 | 0.555 | 0.581 |
| | Within Groups | 107675.9 | 27 | 3987.996 | | |
| **4** | **comparison in serum iron and TIBC in different iron intake amounts** | | | | | |
| | | Sum of Squares | df | Mean Square | F | Sig. |
| serum iron | Between Groups | 880.03 | 2 | 440.015 | 4.699 | 0.011 |
| | Within Groups | 9082.88 | 97 | 93.638 | | |
| TIBC | Between Groups | 56247.53 | 2 | 28123.76 | 4.808 | 0.01 |
| | Within Groups | 567398.5 | 97 | 5849.469 | | |
| **5** | **comparison in serum iron and TIBC in different menstruation days** | | | | | |
| | | Sum of Squares | df | Mean Square | F | Sig. |
| serum iron | Between Groups | 920.227 | 5 | 184.045 | 2.014 | 0.088 |
| | Within Groups | 5848.358 | 64 | 91.381 | | |
| TIBC | Between Groups | 54435.25 | 5 | 10887.05 | 1.536 | 0.191 |
| | Within Groups | 453482.1 | 64 | 7085.658 | | |

# Chapter four

# Discussion

# Chapter four

# Discussion, Conclusion and Recommendations

## 4.1 Discussion

In this cross-sectional study we found that the prevalence of anemia and IDA was lower than the WHO,UNU&UNCEF(2001) estimated that 42% of all women and 52%of pregnant women in developing counters are anemic, with half having IDA.

In this present study, the women`s age (15-47) were categorize into three different groups. The prevalence of IDA in the different group showed that IDA as a serious health problem exists in 15-25 years .In contrast, moderate IDA and severe IDA were the most serious in the same age group. Many studies reported the loss of iron through menstruation; therefore that is why the women in reproductive age need to increase iron intake(0.4-0.5mg/dl/day) (Gibney *et al*.,2007) .But by using statistic test no significant association found between age group and occurrence of anemia(P=0.42). Therefore, we confirm Bharati *et al* (2008) that found the different categories of age among women in reproductive age have no effect on anemia.

In the study of etiological factors, no significant relationship was observed between the number of days of menstrual bleeding and occurrence of iron deficiency anemia (*P*=0.263). This is agreement with the study of Fatin *et al*. (2011) in the study of IDA amonge women at reproductive age in Saudia Arabia which found that, there was no statistically significant relationship between occurance of anemia and the duration of menstruation day. The risk for iron deficiency increased 6 times in women in reproductive age with 6-7 days of menstruation when compared to women with 3 day of menstruation or less.

In our study showed that the most subjects had a high level of education (52%). No association was found between level of education and iron severity of anemia (*P*=0.14) and this was supposed to because understanding about factors related to iron deficiency has already been known about subjects with low level of education. They have it through intensive information, education, communication given by available health services, and information from mass media. Other research done by siti *et al* (2010) in Malaysia, also reported that there was no significance difference between level of education and anemia in pregnant women (*P*=0. 62).The same result was also found in research at Sapa village, Indonesia with (*P*=0. 72).

The resent study showed that most subjects had moderate iron intake consumption (41%). (2) Subject from women with low iron intake consumption has a severe anemia. And significance statistically difference was found between level of iron intake consumption and iron store status (*P*=0.01). Other study revealed similar results that female subjects infrequently consuming of red meat and vegetables (Less than two servings of red meat and vegetables per week) were at increased risk of ID&IDA (Al-Quaiz, 2001).A longitudinal research for 10 years in the united states is done to find out the effect of heme and nonheme iron intake on body store (Garry *et al.*, 2000).No significant association was found between dietary iron and estimated iron store status.

## 4.2 Conclusions

The prevalence of iron deficiency anemia poses significant health problem in women of reproductive age in Khartoum state (27%).

A third of these women are affected by anemia and nearly half of women aged 15-25 are iron deficient.

Infrequent or no consumption iron intake was correlated with IDA. There are some other factors like heavy menstrual blood loss.

Pregnancy and parity levels may account for such an effect.

## 4.3 Recommendations

1. Screening for iron deficiency in high risk groups should be considered.

2. Primary physician education is needed to ensure a greater awareness of IDA and the testing needed to establish diagnosis as well as underline causes.

3. Implementation of iron fortification is recommended. One approach is to fortify a basic food such as cereals and food made from grain, which are foods consumed in substantial quantities by the most women (15-43 years).

4. Daily protocol of iron supplementation is recommended to prevention and treatment of IDA.

# References

## REFERENCES

**Abdalla. A**, (2011), frequency of iron deficiency anemia and thalassemia among anemias Sudanese women of reproductive age.

**AL-Quaiz, J.M.** (2001).Iron Deficiency Anemia: A study Of Risk Factors. *Saudi Med J **22(6)***:490-496.

**Anderson, C.** Aronson, I and Jacobs, P. (2000). Erythropoiesis: erythrocyte deformability is reduced and fragility increased by iron deficiency. *Hematol, 4*:457-460.

**Andrews , N.C**., Greer , J.P., Forester, J and Rodgers G.M.(2009).Iron deficiency and Related Disorders Wintrobe`s Clinical Hematology.12 edition, 810-56,Mary land: Williams and Wilkinsv Ltd,.

**Asakara**, **K.,** Sasaki, S., Murakami. Takahashi, Y., *et al*. (2009). Japan Dietetic students for nutrition and Biomakers Group. Iron Intake dose not Significantly Correlate with Iron Deficiency among Young Japanese Women .*Public Nutr, **12(9):*** 1373-1383.

**Ashesh, B**., Anil. S., Sanjay. S., Sujatha. R. (2016). Iron deficiency anemia in women in reproductive age attending atrtiry care hospitale .Indian J Sci Res7 *(1)*:109-113.

**Aspuru k**, Villa C, Berrnej, O., Herrero, pandlepez, S.G. (2011).Optimal .*Int JGen Med, 4:741*-750.

**Balarajany,** Ramakrishnan U.A., zaltin E, Shankar A. H., Subramanian A.V. (2013). Anemia in Low income and middle income countries .*Lancet,* 378: 0140-6736.

**Barbin, B.J**., Hakimi, M., and Pelletier, D. (2001) An Analysis of Anemia and Pregnancy –related Maternal Mortality .*J nutr*, 131:604-614.

**Barton J.C**., Barton E.H., Bertoli L.F., Gothard C.H., Sherrer J.S. (2000). Intravenous Iron Dextran therapy In patient with iron deficiency anemia and Normal Renal Function who Failed to respond to or did Not tolerate oral Supplementation.*AMJMed,109*:27-32.

**Bermejo F.**, Cronica S., (2006) Deorigen Digestivo. In: PorceJ, Carballo F, Gomollon F, editors. Tratamien de Las enfermedas Gastroenterologicas.2nd edition .Espanola .PP.465-475.

**Bharati, P.,** Som.S. Chakrabarty, S., and Pal., M. (2008). Prevalance of anemia and determination among non-pregnant women in India, *As Pac Pub, 20*:347.

**Birbrair**, A. and Frenette, p. (2016). Niche Heterogeneity in the Bone marrow *An of N Aca of science,* 1370:82-96.

**Brawn**, **D**. Arumsari, E. Pusporini. (2011) Risk Factor of Anemia in School Girl Participant of supplementation on program. *JF nutr, (6*): 74-83.

**Brune, M**. (1986). Iron Less in Sweat. *AJClin Nutr*, *43*:438-43.

**Coad, J** and Conlon C., (2011). Iron Deficiency Anemia in Women: Assessment, Causes and Consequence .*Curropin Clin Nutr Metabcare, 14:625634*.

**Cook J**, **D**. (2005).Diagnosis and Management of Iron Deficiency Anemia .Best.

**Crawley, J.** (2004).Reducing Anemia in Infants and Young Childern in Andemic Countries of Africa: The *J trop med and hyg*, *71*:25-34.

**Crichton. R.** (2009) Iron metabolism: From molecular mechanism to clinical consequence .3rd edition .17-56west Sussex UK: Johonwiley and sons; *PP.141325*.

**Cumana. A** and Godin .I. (2007). Ontogeny of System. *Ann Rev Immunol 25*: 745-785.

**Djazeryery, A.** (2001).Iron status and socioeconomic determinant of the quantity and quality of dietary iron in group of rural Iranian .*East .Med .H J, **7**(4/5)*:652-659.

**Elnicki, D. M**., Shochcor, W.T., Brick, J. E., Beynon, D. (2001).Evaluating the complaint of fatigue in primary care: diagnosis and outcomes. *AmJ Med, 39*:3036.

**Fatin A**., Mamdooh G., Safaa Q., Nadiah B., and Adel A. (2011).Prevalence of iron deficiency anemia among female at university stage. *JMLD, **2**(11)*:PP.5-11.

**Fernandez**, **K.S.,** DeAlarcon, P. A. (2013). Development of Hematopoietic System of-Hematopoiesis.

**Fogelholm, M.,** Suominen, M., Rita, H. (1994).Effect of Low-dose Iron Supplementation in Women with Low serum Ferritin Concentration *Euro J of Clin Nutr, 48*:753-756.

**Garry P. J.** Hunt W. C and Baumgartner R.N. (2000) .Effects Of Iron Intake on Iron Store in Eldery Men and Women: Longitudinal and cross-sectional Result. *J AM Coll Nutr, 19*:262-9.

**Geissler, T.S** and Powers**,** H.J. (2005) Human Nutrition. Edinburgh: Elsevier Churchill Livingstone.

**Georgiades, C.S.,** Neyman, E. G, Francis, I.R. Sneider, M.B and Fishman, E. (2002).Typical and Atypical presentation of extra medullary hemopoiesis .*Amjroentg, **179** (5)*:1239-43.

**Giboney M. J**., Margetts, B. M., Kearney, J. M. and Arab, L. (2007).Public Health Nutrition. Oxford: Blackwell Science.

**Gibson, R. S.** (2005). Principl of nutritional Assessment. (2nd edition) London: oxford university press.

**Godder, A. F**., James, M.W., Mcintyre, S. S and Scotte, B. B. (2011). Guideline for Management of Iron deficiency anemia .*Gut, **60(10)***:1309-16.

**Gupta R.** and Kaila .M. (2004) Diagnosis of Anemia in Expectance Mothers. *Human J of Ecol, **15(1)***:69-7.

**Haider. B.,** Olofin. I., Wang. M, Spiegelmann. D, Ezzati .M, Fawzi .W. Anemia; Prenatal Iron Use, and Risk of Adverse Pregnancy Outcomes (2013): Systematic Review and Meta-Analysis, *BMJ, 346* (F3443).

**Halterman, J. S.,** Kaczorowskim, J.M, Aligne, C.A, Auinger P, Szilagyi, P.G (2001) .Iron deficiency and Congnitive Achievement among School aged Children and Adolescents In United State. *Pediatrics* 107:1381-6.

**Harvey, J. W**. (2000) Microcytic anemia .In Feldman.BF, Zinkl. J. G., Jain. M.C., editor s. Schalm`s veterinary hematology. Philadelphia, PP.200-204.

**Hauke, R.,** Stefano. R, Tarantolo. (2000). Hematopoitic Growth Factor, Labrotory Medicine.

**Hoffbrand, A. V**& Pettit, J.E. (2000).Essential Hematology.4th edition .Oxford Black well science.

**http://** www.repositorysusttech.edu.com /handle 12/6/2010.3:40pm.

**Idris, M. &**Redman ,A.U.(2005).Iron Deficiency Anemia In Moderate To Severely Anemic Patient .*J Aube med cullabbott bad,**17(3)***:108-10.

**Janze, T.G**., Johonson, R. L., Rubenstein, S.D. (2013). Anemia in Emergency Department: Evalution and Treatment. *Emer Med Pract, **15** (11)*:1-15; quiz15-6.

**Kazi .T. Kandhhro**. G, Sirajuddin. G. (2010). Evalution of Iodine, Iron and Selenium Samples biological of Thyroid mother and their newly born in babies. *Early Hum, Devel, 86(10),* 649-655.

**Kushansky, K.** (2006). Lineage-specific hematopoietic growth factors*N Engl .JMed.**354** (*19):2034-45.

**Lacombe, C**and Mayeux, P. (2000).The molecular biology of erytheopoietin. *Nephrol Dial Transport, 14SUPPL 2*:22-8.

**Lee.** A and Okman .M. (2011).Anemia in pregnancy .Hematol OncoL *Clin N Am 25*:241-259.

**Loannou, G.M.,** Spector, J., Scott, K., Rockey, D. C. (2002). Prospective evolution of clinical guideline for diagnosis and management of iron deficiency anemia *Am Med, 113*:281-7.

**Makaron.** A and Josieph, M. (2016).Anemia: Practice, essentials, Pathophisology and etiology.3rd edition.PP.143-665.

**Martius. F. (**2009). Eisenmangelohne Anemia: einheIsses Eisen, Swiss Med.1st edition, Weekly 9,294-299.

**Mateos M.E.,** De Lacruz B.J., Lopez L.E., Valdes M.D., Nogalaes A.C. (2009).Review of Haematology and Biochemistry Para meter to identify Iron.deficiency.2nd edition:761-111.

**McCann, J.** Ames, B. N. (2007).An overview of evidence causal relation between iron deficiency during development and deficits in cognitive or behavioral function.*AMJClinNutr*,***85(4)***:931-945.

**Mehata, A**& Hoffbrand, V. (2000).Hematology at Galance 1est edition.OxfordBlackwellScience.6thedition.

**Milman .N.** Bergholt .T. Eriksen.L., Gradual .N. Pederson. (2005). Iron prophylaxis during pregnancy .*Acta, Gyn Sca, 84*:238-47.

**Mitchel,**Ritchard,Sheppard;Kumar,Viray;Abbas,AbulK;Fausto;Nelson.(2007). Robbins Basic pathology, Philadelphia .8th edition.

**Morrison .J.** kimble .J. (2006). Asymmetric and Symmetric Stem Cell Division in Development and Cancer .*Nature*, ***441****(7097)*:1068-74.

**Morrison, S. J,** and Weissman, I. L., (2000). The long term repopulating subset of hemophilic stem cell is deterministic and ISO lable by phenotype.*Immunity*.***1*** *(8)*:661-73.

**Murray, R. K**., Grinner D, K., Mayes, P.A and Rod well, V.W. (2009).Harpers Biochemistry.25th edition. Appleton &Lange. USA P: 870-871.

**Parslow, T. G.,** Stites, D.P, Terr, A. L and Imboden, J. B. (2008) .Immunology*1st* edition.

**Pennonen, k.,** Irjala.K.RajamaK ,A.(2000).Serum transferrin an it Ratio to serum Ferittin in the diagnosis of iron deficiency anemia .89:1052-10507.

**Raharjo .**B. (2003).Factor–Factor Risko Yang Ber human dengan anemia Pada pekerja perempuan de desa jetis kecamatan sukoharjo kolopaten sukoharjo Tesissermarang.

**Schorr, T. O.**, Hediger M.L. (1994).Anemia and Iron Deficiency Anemia: Complication of data On Pregnancy outcome *Am J clin Nutr, 59- 492s-50s*

**Semba R.** Dand Ramakrishnan U. (2008).In R.D Semba & M. W. Bloem (2nd eds), *Nutr healt dev countries*,(PP.479-505). USA: Humanpress.

**Shayeghi,M.**Latunde,G.O.,Oakhill,J.S.,Laftah,A,H.,Takeuchi,K.,Hallliday,N.(2005).Identification of an intestinal heme transporter *Cel122:789*-801.

**Simth, R. E.** (2010).The clinical and economic burden of anemia. *Am J of Mang Care* 16, 59-66.

**Siti, A.R**., Rosnah .S. Rahmah, M.A. (2010).Prevalen Anemia Semasa Mengandung Dan Factor-Factor Mempengaruhinya Di Johor Bahru *M J P H Med **10**(1)*:70-83.

**Stevens, G.** A, Finucane , M. M, Flaxmen, S. R., Paciorek, C. J, &Regil, L. D. (2013) .Global ,Regional, and National Trend in Hemoglobin Concentration and Prevalence of Total and Severe Anemia In Children and Pregnant Women For 1995-2011:Systematic Analysis of Population .Representative Data .*The lanc glob. Health, 1*: e 16-e25.

**Stoltzfus R, J**.,Mullany L ,Black R.E.,(2004).Iron Deficiency Anemia .In : Ezzati M, Lopez A.D,MurryCJL.editors. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. Geneva: World Health organization: 163-210.

**Tavian .M**.,biasch, K., Sinka L., Vallet, J. and Peault B. (2010).Emberyonic Origin of Human Hematopoiesis *Int J Dev bio* 54:1061-1056.

**Tefferi, A.** (2003).Anemia in adult: Acontemporary apporch to diagnosis. *Mayo clinc Pro, 78*:1274-1280.

**Verdon .F**, Burnard .B, Stubi, C. L, Graf, F. M, Michaud, A. (2003). Iron Supplementation for Unexplained Fatigue in Non –Anemic Women: 326:1124.

**Weather all. D. G** and Chrishhatton. (2003). Oxford Text book 5th edition, London.

**World Health Organization.** (**WHO).** (2012).Iron Deficiency Anemia: Assessment, Prevention and control.

**Weiss. D, G.** (2010) Iron and copper deficiencies and disorder of iron metabolism .6thedition.Ames: Black well Publishing; PP.167-171. (55.6%).

**World Health Organization (WHO)**. (2004).Micronutrient deficiency: Batting iron deficiency anemia: The challenge.

**World Health Organization (WHO).** (2005).Iron deficiency Anemia: Assessment, Prevention and control.

**World Health Organization (WHO)**. (2008).World prevalence of anemia 1993-20\05.WHO Global database of anemia .Geneva. World Health Organization.

**World Health Organization (WHO).** (2011) .The global prevalence of anemia.

**Wintrobe, M. M** and Lee, G. R. (2000). Wintrobe Clinical Hematology 10thed. Baltimore Md: Williams& Wilkins.

# Appendixs

## Appendix I

### Sudan University of Science & Technology
### Collage of Medical Laboratory Science

### Assessment the Prevalence of Iron Deficiency Anemia among Female in Reproductive Age (15-46) In Khartoum State, Sudan

### Questionnaire

**Women ID……..**

| | | |
|---|---|---|
| 1 | Age | |
| 2 | Marital status | Married |
| | | Not married |
| 3 | Education level | Illustrate |
| | | Primary |
| | | Secondary |
| | | University |
| | | Higher education |
| 4 | Are you pregnant? | Yes |
| | | No |
| | | |
| 5 | Duration of menstruation days | 2-7 [] |
| | | >7 [] |
| 6 | Red meat consumption | High |
| | | Moderate |
| | | Low |

-Investigations: CBC, Serum iron, Total Iron Binding Capacity.

-After understanding the content of this questioner I agree ……to collect this sample.

Date/……

## Appendix II

**إقرار موافقة**

**فقر الدم عند النساء في فترة الخصوبة بولاية الخرطوم**

هذه دراسة تعمل علي قياس معدل إنتشار انيميا نقص الحديد عند النساء ونقوم بجمع المعلومات وعينات دم وريدي منك لعمل الفحوصات اللازمة وفي حالة حدوث اي مضاعفات ارجو الرجوع الي .

بعد الفهم التام لمحتوي الاستبيان اوافق علي جمع العينات .

إسم المتبرع/ ..... العنوان/ .....

التوقيع/.....

إسم الباحث/..... العنوان/ .....

التاريخ / /

## Appendix III

## Laboratory Requirement

1. Reagents:

- Basic magnesium carbonate, Mg Co3.

- Saturating solution (100m.ml fe/l)

-Protein precipitant.

-Chromgen solution.

-Iron-free water.

-Iron standard 80mmol/l.

-Sysmex solution.

2. Equipments:

- Sterile disposable plastic syring.

-70% ethanol.

-Cotton.

-Blood container.

-Centrifuge.

-Spectrophotometer.

-System Kx-21.