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Hepcidin Status among Iron Deficient Anemic Pregnant Women in Gaza strip: A Case Control Study

مستوى الهيبسدين لدى النساء الحوامل المصابات بانيميا نقص الحديد في قطاع غزة: مجموعة مرضية – مجموعة ضابطة

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إقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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مستوى الهيبسدين لدى النساء الحوامل المصابات بأنيميا نقص الحديد في قطاع غزة: مجموعة مرضية- مجموعة ضابطة

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وبعد المناقشة التي تمت اليوم الثلاثاء 01 رجب 1438هـ، الموافق 2017/03/28 الساعة العاشرة والنصف صباحاً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله ولزوم طاعته وأن يسخر علمها في خدمة دينها ووطنها.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

Abstract

Background: Hepcidin, a peptide hormone composed of 25 amino acids. Hepcidin is synthesized mainly in the liver. Iron deficiency anemia (IDA) is common during pregnancy and is associated with higher maternal morbidity and mortality in Gaza strip. Understanding of hepcidin hormone and its role in iron metabolism could lead to new indicators for earlier detection of cases with IDA.

Objective: To assess hepcidin status among IDA pregnant women and its relationship with some biochemical variables in Gaza strip.

Materials and methods: A case control study this study comprised 45 IDA pregnant women and 45 healthy pregnant women. Questionnaire interviews were applied among the study population. Serum hepcidin and ferritin were measured by ELISA, iron and TIBC were determined photometrically. Complete blood count (CBC) was also performed. Transferrin and transferrin saturation were calculated. An approval was obtained from local ethical committee to conduct this study. Overall data were computer analyzed using SPSS.

Results: The mean level of serum hepcidin, iron, transferrin saturation, and ferritin in cases were significantly lower than that in controls (2.6±4 ng/ml, 63.2±25.3 µg/dl, 15.6±8.0% and 8.0±9.7 ng/ml versus 7.5±7.3 ng/ml, 77.7±22.9 µg/dl, 23.5±8.0% and 15.4±14.3 ng/ml respectively with p=0.000). The Pearson correlation test showed positive significant correlations between hepcidin levels and serum iron, ferritin, and transferrin saturation (r=0.547, p=0.000; r=0.558, p=0.000 & r=0.577, p=0.000 respectively). On the other hand, negative correlations were showed with TIBC and transferrin (r=-0.551, p=0.000 & r=-0.526, p=0.000) respectively. The average values of RBC, Hb, HCT, MCV, MCH, and MCHC were significantly lower among IDA pregnant women (3.3±2.4, 9.7±0.8, 29.4±2.3, 76.6±4.8, 25.6±2.2 & 33.2±1.5 respectively) compared to controls (4.0±0.3, 11.8±0.6, 34.7±2.0, 86.3±3.3, 29.4±1.3 & 34±0.9; p=0.000) respectively. RDW was significantly higher in cases vs. controls (16.6±2.4, 13.7±0.6; p=0.000).

Conclusions: Hepcidin hormone was lower in IDA pregnant women than healthy pregnant women. Thus it is recommended to carry out further studies to evaluate the role of hepcidin in the diagnosis of IDA among different gestational women.

Keywords: Hepcidin, Serum Iron, Ferritin, Iron deficiency anemia, Gaza strip**.**

ملخص الدراسة

المقدمة: الهيبسدين هو هرمون ببتيدي يتكون من 25 حمض اميني ويصنع الهيبسدين اساسا في الكبد وهوشائع اثناء الحمل ويرتبط مع ارتفاع معدلات الاعتلال والوفيات النفاسية في قطاع غزة. نقص الحديد هو السبب الأكثر شيوعا لفقر الدم عند النساء الحوامل. فهم هرمون الهيبسدين ودوره فى ايض الحديد قد يؤدى الى ايجاد مؤشرات جديدة للكشف المبكر عن الحالات المصابة بأنيميا نقص الحديد

الهدف: تقييم مستوى الهيبسدين لدى النساء الحوامل المصابات بانيميا نقص الحديد في قطاع غزة وعلاقته ببعض المتغيرات البيوكيميائية.

الطرق والأدوات: منهج الدراسة (مجموعة مرضية – مجموعة ضابطة) المجموعة المرضية تحتوي على 45 امراة حامل مصابة بانيميا نقص الحديد والمجموعة الضابطة تحتوي على 45 من النساء الحوامل الغير مصابين بانيميا نقص الحديد وقد تم الحصول على البيانات المستخدمة في الدراسة من خلال المقابلة المباشرة وتم قياس مستوى الهيبسدين والفرتين بواسطة تقنية **ELISA**، كما تم تحديد مستوى الحديد والحديد المرتبط وكذلك مكونات الدم .وتم حساب نواقل الحديد والنواقل المشبعة. وقد تم اخذ البعد الاخلاقي للدراسة حيث تم الحصول على موافقة من لجنة هلنسكي المحلية. تم تحليل البيانات والنتائج باستخدام البرنامج الاحصائي المحوسب SPSS.

النتائج: كان متوسط مستوى الهيبسدين والحديد ومخزون الحديد في الدم ونواقل الحديد المشبعة اقل وذات دلالة احصائية في المرضى منها في المجموعة الضابطة ولقد اظهرت النتائج وجود علاقة طردية بين مستوى هرمون الهيبسدين والحديد ومخزون الحديد والنواقل المشبعة في الدم وكانت نتيجة مستوى الحديد المرتبط ونواقل الحديد اعلى بدلالة احصائية في المجموعة المرضية منها في المجموعة الضابطة. اظهرت النتائج أيضا وجود علاقة عكسية ذات دلالة احصائية بين مستوى هرمون الهيبسدين والحديد المرتبط ونواقل الحديد وكان متوسط مستوى كرات الدم الحمراء والهيموجلوبين والهيماتوكريت ومتوسط حجم الخلية ومتوسط هيموجلوبين الخلية ومتوسط تركيز هيموجلوبين الخلية اقل في المجموعة المرضية منها في المجموعة الضابطة وقد كانت النتائج ذات دلالة احصائية. وكان مستوى عرض توزيع كرات الدم الحمراء (RDW) اعلى في المجموعة المرضية منها في المجموعة الضابطة وقد كانت النتيجة ذات دلالة احصائية وقد اظهرت النتائج ايضا وجود علاقة طردية بدلالة احصائية بين مستوى هرمون الهيبسدين وكرات الدم الحمراء والهيموجلوبين.

الاستنتاج: تركيز مستوى هرمون الهيبسدين اقل عند النساء الحوامل المصابات بانيما نقص الحديد عن النساء الحوامل الغير مصابات بانيما نقص الحديد ولوحظ ان هناك علاقة طردية بين مستوى هرمون الهيبسدين والحديد ومخزون الحديد ونواقل الحديد المشبعة وعكسية بين الهيبسدين والحديد المرتبط وناقل الحديد. من المستحسن اجراء مزيد من الدراسات لتقييم دور الهيبسدين في تشخيص أنيميا نقص الحديد بمراحل مختلفة عند النساء الحوامل.

الكلمات المفتاحية : الهيبسدين، الحديد في الدم ،مخزون الحديد،انيميا نقص الحديد، قطاع غزة.

Dedication

I dedicate this work:

To my father and mother

To my beloved husband for always supporting me

To my children Dana and Abdullah

To my sisters and brothers

To all my teachers who supported me

To all my friends and colleagues who were directly or indirectly involved in the research

To my university The Islamic University of Gaza which is continuously improving the research

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At the end, I am very grateful to every person who participated and helped me to complete this study.

Esraa M. Elnabaheen

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List of Abbreviations

BMI	Body Mass Index
BMP6	Bone morphogenetic protein6
CAD	Coronary Artery Disease
CBC	Complete Blood Count
CDC	Center for Disease Control
CHC	Chronic Hepatitis C
DIOS	Dysmetabolic Iron Overload Syndrome
DMT1	dimetal transporter 1
EDTA	Ethylene diamine tetra Acetic Acid
ELISA	Enzyme linked immuno-sorbent Assay
HAMP	Hepcidin Antimicrobial Peptide
HB	Hemoglobin
HCT	Hematocrit concentration
IDA	Iron Deficiency Anemia
IL-6	Interleukine-6
IRIDA	Iron-Refractory Iron Deficiency Anemia
JH	Juvenile Hemochromatosis
LEAP-1	Liver Expressed Antimicrobial Protein-1
MCH	Mean Cell Hemoglobin
MCH	Mother and Child Health
MCHC	Mean Cell hemoglobin concentration
MCV	Mean Cell Volume
NHIOD	Non Hereditary Iron Overloading Disease
NMR	Nuclear Magnetic Resonance
RDW	Red Cell Distribution Width
SCD	Stem Cell Disease
SCT	Stem Cell Transplantation
SPSS	Statistical Package of Social Science
TIBC	Total Iron Binding Capacity
TSAT	Transferrin Saturation Test
WHO	World Health Organization

Chapter one
Introduction

Chapter one
Introduction

1.1 Overview

Iron is considered as an essential element for virtually all living organisms. Iron is found in meat, poultry and plant-based foods as well as in supplements. There are two types of iron in foods (Fleming and Sly, 2002). 1) Heme iron is the type that the body absorbs best. It is found in beef, chicken, turkey, and other types of meat. 2) Non heme iron is the other type, which is found in plant sources that is absorbed partially. Iron participates in a wide variety of metabolic processes, including oxygen transport from the lungs to the tissues by hemoglobin in red blood cell and electron transport.

In pregnant women significant changes can be observed in iron metabolism. Pregnant women needs about twice the amount of iron. Iron is an essential element for pregnant women who have an increased physiological need for it. This causes an increase in the demand for iron during pregnancy, therefore serum iron levels decrease, but the total iron binding capacity increases. Ferritin levels demonstrate a downward slope in both plasma and tissue; about 50% of pregnant women don't get enough of this important mineral (Ervasti, Kotisaari, Heinonen and Punnonen, 2007).

Iron deficiency is a common cause of anemia in pregnancy which is associated with maternal and foetal problems such as preterm labor and maternal infections. Iron supplementation during pregnancy is usually based on hemoglobin values, although physiological hemodilution in pregnancy often leads to reduced hemoglobin values. Thus, new indicators are necessary for earlier detection of iron deficiency anemia (IDA) in pregnancy (Goepel, Ulmer and Neth, 1988).

Recent studies have evaluated the use of hepcidin as a biomarker for the regulation of iron metabolism. Human Hepcidin is a 25-amino acid peptide that is secreted by the liver and excreted by the kidneys, and it is considered to be a major regulator of iron metabolism and the anemia that is associated with chronic inflammation (Park, Valore, Waring and Ganz, 2001). Hepcidin was first discovered in human urine and serum in the year 2000. The peptide was initially reported as Liver-Expressed Antimicrobial Protein-1 (LEAP-1), and later became known as hepcidin (Krause et al., 2000).

Hepcidin inhibits iron transport by binding to the iron channel ferroportin, which is located on the surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (Rossi, 2005). It regulates iron metabolism by inhibiting duodenal iron absorption at the level of the intestinal epithelium, and by affecting mobilization of iron from liver and spleen (Ganz, 2005). Hepcidin activity is also partially responsible for iron sequestration seen in anemia in chronic disease (Weiss and Goodnough, 2005). In addition, this hormone regulates the transfer of iron through the placental syncytiotrophoblast during pregnancy (Bastin et al., 2006). Despite the central role of hepcidin in the metabolism of iron, limited data are available that associate hepcidin levels with measures of iron status, inflammation, and anemia among pregnant women (Evans et al., 2011).

In recent years, knowledge has increased about hepcidin and its role in the absorption and movement of iron in the body which has started to provide a more functional view of iron metabolism. Hepcidin is a pivotal regulator of iron metabolism because it controls the efflux of iron from enterocytes, hepatocytes, and macrophages by internalization and degradation of the iron exporter (ferroportin), and also regulates the plasma iron level. Hepcidin is up regulated in response to an increase of body iron stores or the onset of infection and is down regulated by anemia or hypoxia, while it is also an acute phase reactant induced by inflammation that shows antimicrobial activity. The discovery of hepcidin and further understanding of how it inhibits the movement of iron and its regulation may eventually help clinicians better evaluate a patient's iron status and may assist in more effective, efficient treatment for anemia of chronic diseases (Knutson, 2010).

1.2 Objectives of the Study

1.2.1 General Objectives

To investigate hepcidin status among iron deficient anemic (IDA) pregnant women in the third trimester in Gaza strip.

1.2.2 Specific Objectives

1. To determine and evaluate the effect of serum hepcidin level in IDA pregnant women patients and controls.
2. To investigate the possible relationship between hepcidin hormone and IDA.
3. To investigate the possible relationship between hepcidin hormone and the following parameters: (serums iron, serum ferritin, TIBC, CBC indices, transferrin and transferrin saturation).
4. To identify the risk factors associated with IDA.

1.3 Significance

Iron deficiency anemia is a common nutritional disorder in the Gaza Strip, where the most affected group by this disease is pregnant women. It is associated with maternal and foetal problems such as preterm labor, maternal infections, relative risk of preterm birth, low birth weight and prenatal mortality. Understanding of hepcidin hormone and its role in iron metabolism could lead to new indicator for earlier detection of cases with IDA. Moreover the analysis of hepcidin together with other tests will help in choosing the best therapy for anemia. According to our knowledge, this study will be the first one that focuses on hepcidin hormone among pregnant women and its association with IDA in the Gaza Strip.

Chapter two

Literature Review

Chapter 2
Literature Review

2.1 Iron

Iron plays a significant role in biology, creating complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are the commonly responsible oxygen transporting proteins in vertebrates. Iron is also the metal at the dynamic place of many vital redox enzymes dealing with cellular respiration and oxidation in plants and animals (Anderson, Darshan, Wilkins and Frazer, 2007).

Excessive iron can badly affect the body. Taking lots of iron supplements can cause iron poisoning. Some people have an inherited disease called hemochromatosis, which causes too much iron to build up in the body. The body requires the right quantity of iron. If there is lack in iron, a person may grow IDA. Different causes can lead to a decrease in iron levels including blood loss, poor diet or an incapability to absorb enough iron from foods. People who have higher danger of having too little iron are young children and women who are pregnant (Anderson et al., 2007).

2.2 Anemia

Anemia is commonly defined according to hemoglobin levels, which may differ according to a lot of factors most significantly age, gender, and ethnicity. Any level below 13 g/dL for males and below 12 g/dL for females is considered abnormal (Thum and Anker, 2007). Hemoglobin levels which are less than 11 g/dL at any time during pregnancy are considered abnormal. As soon as anemia is recognized, the opportunity of iron deficiency should be considered (Shill et al., 2014).

2.3 Iron deficiency anemia (IDA)

2.3.1 Definition

Generally, IDA is defined as a reduction in the oxygen carrying capacity of the blood and considered as the main reason for microcytic hypochromic anemia. This remarkably reduces the hemoglobin per deciliter of blood and volume of packed red blood cells (hematocrit), or the number of erythrocytes. Abnormalities in red blood cell indices on

complete blood count typically come before the progress of lowered hemoglobin levels. Iron shortage usually grows slowly over time, and may not be symptomatic, or clinically clear. Once iron stores are completely exhausted, iron convenience to the tissues decline leading to symptomatic anemia (Burke, Leon, and Suchdev, 2014).

IDA is the most prevalent medical difficulties of pregnancy, mainly because of growth of plasma volume without normal growth of maternal hemoglobin mass (Camaschella, 2015) women with poor diet histories, recurrent conceptions or records of previous iron reduction are mostly at risk. Woman's nutritional status prior and during pregnancy can significantly affect her own health and that of her unborn child. Generally, there are many factors that affect women's ability to attain good prenatal nutrition including the following:

- General nutritional status prior to pregnancy: the proper eating through life, not just during pregnancy can lead to good prenatal nutrition, even though pregnancy may motivate a woman to enhance poor eating routines. The outcome of the pregnancy can be affected by any decrease of nutrition at conception or during the early prenatal period.
- Maternal age: a pregnant adolescent must get the nutritional needs for her own growth as a teenager in addition to the nutritional needs during pregnancy.
- Maternal parity: mother’s nutritional needs and the result of her pregnancy are affected by the number of pregnancies and intervals she got through, besides that, the nutritional posture does affect her fetus. Factors affecting fetal well-being are interconnected, but nutrient deficits alone can produce measurable effects on evolution of the developing fetus.
- Socioeconomic level: poor families can’t afford the same foods as higher income families can. Thus, poor pregnant women with low incomes are usually at risk for insufficient intake of nutrients.
- Education level: familiarity and knowledge about the main components of a balanced diet is necessary .Often educational level connected to economic grade, but even people on very inadequate incomes can prepare well- balanced meals if their knowledge of nutrition is sufficient.
- Psychological factors: the nutritional well-being is directly affected by emotions and the expectant woman's attitudes and feelings about her pregnancy may affect

her food consumption. An unhappy woman who does not desire to be pregnant may manifest these feelings by loss of appetite or by an excessive eating of certain foods (Rainville, 1998).

2.3.2 Causes of IDA

Generally, IDA among women could happen because of many factors. These include:

1. Deficit consumption or reduction of iron; this contains dietary deficiency and gastrointestinal disturbances such as diarrhea.
2. Extra request such as frequent, many or multiple pregnancies and in this case, iron stores are low in women suffering a short period (less than 2 years) between pregnancies or those from low socioeconomic communities.
3. Chronic infection, especially of the urinary tract.
4. Severe or chronic blood loss, such as menorrhagia (heavy periods), bleeding hemorrhoids, or ante-partum or postpartum hemorrhage.
5. Women who were using intrauterine device (IUD) may be deficient in iron because of the excessive blood loss with menstrual flows, while those who have been taking oral contraceptives have minor risk for getting anemia (Burke et al., 2014).

2.3.3 Symptoms of IDA

IDA has several symptoms and signs including: pallor of the mucous membranes, exhaustion, general weakness, reduced appetite, dizziness and fainting, headache, shortness of breath, increased heart rate (tachycardia) and palpitations (Coad and Conlon, 2011).

2.3.4 Tests for diagnosis of IDA

Many tests are available to diagnose IDA. They can help approve a diagnosis, search for a cause, and discover how severe the condition is.

2.3.4.1 Complete Blood Count

The earliest test used to detect anemia is a CBC. The CBC scales many parts of the blood containing hemoglobin and hematocrit levels. Hemoglobin is an iron-rich protein in red blood cells that carries oxygen to the body. Hematocrit is a measure of how much space red blood cells occupy in the blood. A low level of hemoglobin or hematocrit is

considered as a sign of anemia. The CBC also tests the number of red blood cells, white blood cells, and platelets in the blood. Abnormal results of the test may be an evidence of infection, a blood disorder, or another condition. Lastly, the CBC looks at mean corpuscular volume (MCV). MCV is a measure of the normal size of red blood cells. The results may be a sign as to the cause of anemia. For example, red blood cells are usually smaller than normal in IDA.

2.3.4.2 Serum iron

Serum iron measures the quantity of flowing iron that is bound to transferrin. When there are concerns about iron deficiency, clinicians ask for serum iron test because iron deficiency can cause anemia and other problems. Transferrin is a molecule produced by the liver that binds one or two iron (III) ions, i.e. ferric iron, Fe^{3+}; transferrin is important if kept iron is to be moved and used. Most of the time, around 30% of the available sites on the transferrin molecule is filled. To measure the iron molecules that are bound to transferrin, and flowing in the blood, the test for serum iron uses blood drawn from veins. This is a significant part of the diagnostic procedure for conditions such as IDA, Anemia of chronic disease and Haemochromatosis (Baker and Greer, 2010).

2.3.4.3 Total Iron-Binding Capacity

Transferrin iron-binding capacity is a medical laboratory test that measures the blood's capacity to bind iron with transferrin. It is done by drawing blood and measuring the maximum quantity of iron that it can carry, which indirectly measures transferrin (Yamanishi et al., 2003) since transferrin is the most active carrier. TIBC is cheaper than a direct measurement of transferrin (Kasvosve et al., 2002). The TIBC should not be muddled with the UIBC, or "unsaturated iron binding capacity ". The UIBC is calculated by deducting the serum iron from the TIBC (Yamanishi et al., 2003).

2.3.4.4 Serum ferritin

The ferritin test is used to measure the level of ferritin, the prime iron storage protein in the body. Ferritin’s shape is like a hollow sphere that allows the entry of a multiple amount of iron for storage (as ferric hydroxide phosphate complexes). Ferritin’s job is to keep the iron in a non-toxic form, to deposit it in a safe form, and to move it to areas where it is needed. Ferritin levels can also be used to indirectly measure the iron levels

in the body. High levels of ferritin can indicate an iron storage trouble, such as hemochromatosis, or a chronic disease process. While low levels of ferritin can indicate an iron deficiency, the thing that can cause anemia (Seckback, 1982).

2.3.4.5 Transferrin saturation

Transferrin saturation can be abbreviated as TSAT and measured as a percentage because it is a medical laboratory value. It is the rate of serum iron and total iron-binding capacity of the transferrin that is obtainable to bind iron. This value states to clinician how much serum iron is actually bound. For example, a value of 15% means that 15% of iron-binding sites of transferrin are being occupied by iron (Powell et al., 2011).

2.3.5 Effects of IDA on the mother and infant

During pregnancy, IDA harmfully affects the maternal and fetal well-being, and is connected to enlarged morbidity and fetal death. Affected mothers often suffer breathing difficulties, palpitations, tiredness, fainting, and sleep difficulties. Women who suffer from iron deficiency anemia may not show any symptoms, but they are more liable to infection, may tire easily, with increased chance of preeclampsia and postpartum hemorrhage, and endure poorly even minimal blood loss during birth. Curing of an episiotomy or a cut usually postponed and if the anemia is severe (Hb less than 6g/dL), cardiac failure may follow. On the other hand, there is clue of enlarged risk of low birth weight (Low birth weight/ less than 2.500g). Iron deficiency anemia is connected with a higher incidence of low-birth weight, infant's preterm birth, stillbirth, pre-maturity, and neonatal death in infants of women who suffer severe iron deficiency (maternal Hb less than 6 g/dL). Infants are not iron deficient at birth because of the dynamic transport of iron across the placenta, even when maternal iron stores are low. These babies do have lower iron stores and they are at enlarged risk for increasing iron deficiency during infancy (Lee, Zaffke, and Baratte-Beebe, 2004).

2.3.6 Prevalence of IDA worldwide

IDA is a serious public health problem affecting more than 800 million people in the world, Approximately 50% of cases of anemic are considered to iron deficiency (Stevens et al., 2013). It is considerably more prevalent in the developing regions

(59.0%) than in the industrialized world (14.0%). Previous studies on IDA have revealed a prevalence of 39.7% in Kuwait (DeMaeyer et al.,1989), 78.0% in Liberia (Jackson and Lantham, 1982) 73.9% in Guyana(Johnson, Latham and Roe, 1982), 61.0% in Jamaica (Simmons et al., 1982), 50.0% in Bahrain (Aldallal, 1984), 22.1% in Egypt (El-Rafie et al., 1990) and 19.8% in Northern Ireland (Strain, Thompson, Barker and Carville, 1990).

2.3.7 Prevalence of IDA among women in Palestine

In the Gaza Strip, IDA (Hb< 11g/dl) among pregnant women who attended Government MOH clinics in 2003 reported to be 20.9%. However, a higher percentage (38.3%) reported among pregnant women attending UNRWA antenatal clinics. In 2002, a study conducted in the West Bank area of Palestine showed that the incidence of anemia seems to increase with age and women aged 40-49 are four times more likely to suffer from anemia than women did in the adolescent age. Childbirth and close birth spacing most likely affect the incidence of anemia. According to the Palestinian Bureau of Statistics, a middle child born within 18 months of an older and a younger sibling was significantly more likely to become anemic. The report also showed that anemia increases with number of pregnancies, reaching 48% in women with 11 or more pregnancies. The prevalence of anemia among women aged 15-49 years taking iron was only 7.1% (WHO, 2009).

2.3.8 Treatment of IDA

The most common way of treatment of IDA is the oral iron supplementation and the dose of it depends on severity of condition. Oral iron preparations given prophylactically contain one of the iron salts, either alone or in combination with folic acid. Common iron preparation contains ferrous sulphate, and ferrous gluconate. Data offered in shows the most common obtainable iron salts in the market; however, ferrous sulphate is the most common because it used by Palestinian ministry of health and delivered by mother and child health (MCH) care centers (Stoltzfus and Dreyfuss 1998). Iron insufficiency anemia treated with a daily ferrous iron supplement of 60-120 mg. When the hematocrit turns into normal for the stage of pregnancy, the dose of iron reduced to 30 mg per day. Iron can also be given intramuscularly or intravenously thereby bypassing the gastrointestinal tract. This can be useful in cases where woman

are unable to take, tolerate or absorb oral preparations. Intra-muscular iron is given in the form of iron sorbitol. Iron dextran is given as total dose intravenous infusion. Blood transfusion is seldom used to treat IDA in pregnancy, but it may be considered where there is an insufficient amount of time to treat severe anemia prior to birth (Bothwell, 2000).

2.4 Hepcidin Hormone

2.4.1 History

The peptide was firstly named LEAP-1, which stands for Liver-Expressed Antimicrobial Protein. Later, a peptide connected with inflammation was discovered (Krause et al., 2000) and named "hepcidin" after it was detected that it was produced in the liver ("hep-") and seemed to have bactericidal properties ("-cide" for "killing") (Park, Valore, Waring, and Ganz, 2001). Although it is mainly synthesized in the liver, smaller amounts are synthesized in other tissues such as fat cells (Bekri et al., 2006). Hepcidin was first discovered in human urine and serum in 2000 (Kemna, Tjalsma, Willems and Swinkels, 2008). After this discovery, researchers revealed that hepcidin production in mice rises in conditions of iron overload as well as in inflammation. In experiment on genetically modified mice, the mice engineered to over express hepcidin, but they died shortly after birth with severe iron deficiency, again this suggesting an essential and not redundant role in iron regulation. The first evidence that linked hepcidin to the clinical condition known as the anemia of inflammation, and it came from the lab of Nancy Andrews in Boston, where researchers tested tissues from two patients with liver tumors with a severe microcytic anemia that did not respond to iron supplements. The tested tumor tissue seemed to be overproducing hepcidin, and contained large amounts of hepcidin mRNA. By removing the tumors surgically, the anemia was healed. These discoveries advised that hepcidin adjusts the absorption of iron into the body (Kemna et al., 2008).

2.4.2 Structure, expression and homeostasis

Hepcidin is considered as a significant hormone that is involved in the control of iron homeostasis in the body (Figure 2.1). Physiologically, hepcidin is controlled by inflammation, iron stores, erythropoiesis and hypoxia. The organizing of hepcidin that

expressed by iron is a complex process that needs the organization of many proteins, containing bone morphogenetic protein 6 (BMP6), matriptase-2, transferrin receptor 2, neogenin, BMP receptors, and transferrin. Misregulation of hepcidin is found in a lot of disease states, such as the anemia of chronic disease, anemia, iron deficiency, iron refractory, hemochromatosis, hereditary cancer, and useless erythropoiesis, such as β-thalassemia. Thus, the organizing of hepcidin is the subject of interest for the improvement of the harmful effects of either iron deficiency or overload (Zhao, Zhang and Enns, 2013). The human hepcidin gene (HAMP) is located on chromosome 19q13.1 (Kemna et al., 2008). HAMP gene expression was discovered primarily in the liver, but also in heart, tonsils, salivary gland, prostate gland, brain, lung, and trachea (Krause et al., 2000). HAMP encodes a precursor of hepcidin –preprohepcidin, which is 84 amino acids protein; there are three forms of hepcidin: 25aa, 22aa, 20aa peptide. ALL three forms are noticeable in urine but only hepcidin -25 and hepcidin -20 are present in human serum. The construction of hepcidin -25 which is the major form of hepcidin comprises eight cysteine residues connected by disulfide bonds (Rossi, 2005).

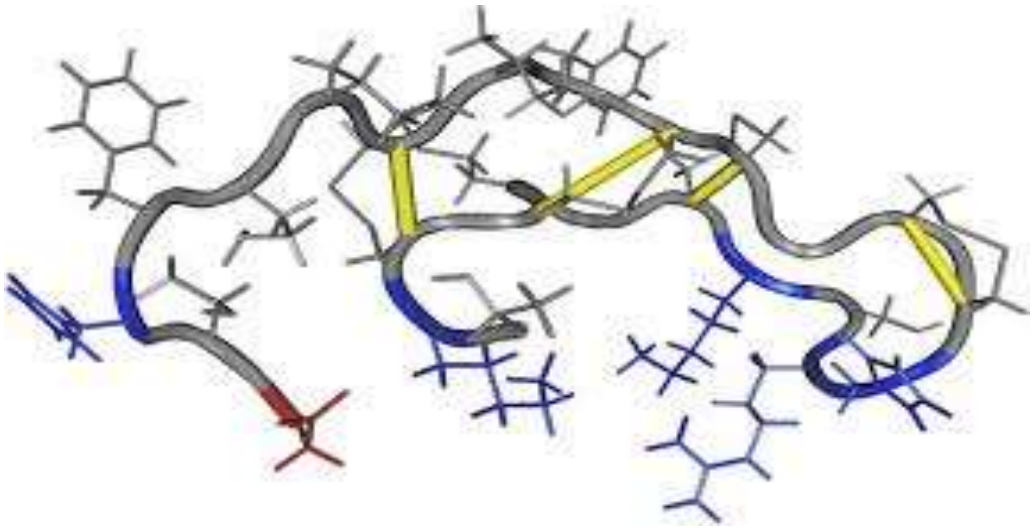

Figure (2.1): NMR structure of hepcidin. The spatial segregation of charged residues is clearly seen in this view, as is the vicinal disulfide bond in the turn (Jain, 2009).

2.4.3 Clinical Significance

There are many diseases where inability to sufficiently absorb iron can lead to iron deficiency and iron deficiency anemia. The treatment will count on the hepcidin levels that are present, as oral treatment will be improbable to be efficient if hepcidin is

preventing enteral absorption, in such cases parenteral iron treatment would be suitable. Studies have discovered that measuring hepcidin would be useful to create optimal treatment. Hepcidin is considered as a key role in the clinical significance of disorders of iron homeostasis. Hepcidin deficiency is the primary cause of many of the most common genetic iron overload conditions, such as sideroblastic anemia, thalassemia intermedia, hereditary hemochromatosis and myelodysplastic syndrome. These disturbances are featured by uncontrolled iron absorption that leads to the accumulation of iron in the liver and other tissues that can lead to organ failure and sometimes death. In cases when hepcidin is in excess, iron absorption is repressed and iron is seized in macrophages, the thing that can lead to iron-restricted erythropoiesis and anemia whose danger is proportional to the level and duration of hepcidin over expression (Bregman et al., 2013).

2.4.4 Mechanisms of hepcidin action

Hepcidin is commonly known as iron regulatory hormone; so generally, hepcidin causes a reduction in serum iron. The mechanism of hepcidin action relies on hepcidin interactions with ferroportin. Ferroportin is the only known mammalian cellular iron exporter, which is expressed on the surface of reticuloendothelial macrophages, placenta cells, duodenal enterocytes, and hepatocytes. Hepcidin regulates post translationally ferroportin expression and binds to ferroportin and causes its internalization and degradation in end lysosomes, the thing that prevents the iron transport via ferroportin. When iron stores are high, increased hepcidin expression blocks intestinal iron absorption and release recycled iron from macrophages and its transport across the placenta. On the other hand, when iron stores are insufficient or low, hepcidin production is restrained. By adjusting hepcidin expression, organism can control plasma iron level and keep iron metabolism homeostasis (Kemna, Tjalsma, Willems, and Swinkels, 2008).

2.4.5 Hepcidin regulation

Iron organizes hepcidin homeostasis. Rises in iron levels in the plasma and iron storage stimulate the manufacturing of hepcidin, which prevents iron absorption from the diet and its further storage (Ganz et al., 2008). Production of hepcidin is restrained in the case of iron deficiency; the feedback loop between iron and hepcidin should confirm the

suitable physiological concentration of iron in the plasma. Hepcidin construction is also organized by the erythropoietin process, whose essential activity is featured by high iron consumption. (Pak et al., 2006). Hepcidin is high during inflammation and/or infection. This can cause iron dysregulation with hypoferremia and anemia connected to inflammatory disease (Ganz, 2006).

Recently, a study has discovered that: 1) hepcidin directly binds to ferroportin, 2) the binding of hepcidin leads ferroportin to be internalized and degraded, and 3) the lack of ferroportin from the cell membrane ablates cellular iron export. This mechanism is adequate to clarify the organizing of iron absorption, because absorptive enterocytes only accomplish their job for 2 days before being shed from the tips of the villi into the intestinal lumen. Therefore, the transport of iron by ferroportin across the basolateral membrane decides whether the iron is transported to plasma transferrin or removed from the body with shed enterocytes. When iron stores are sufficient or high, the liver produces hepcidin, which circulates to the small intestine. There, hepcidin makes ferroportin to be internalized, blocking the sole pathway for the transfer of iron from enterocytes to plasma. When iron stores are insufficient, hepcidin construction is blocked, ferroportin molecules are displayed on basolateral membranes of enterocytes, and there they move iron from the enterocyte cytoplasm to plasma transferrin (Figure 2.2). Likewise, the hepcidin-ferroportin interaction also clarifies how macrophage recycling of iron is organized and accounts for the characteristic finding of iron-containing macrophages in inflammatory states characterized by high creation of hepcidin. In the existence of hepcidin, ferroportin is internalized, iron export is jammed, and iron is trapped within macrophages (Nemeth et al., 2004).

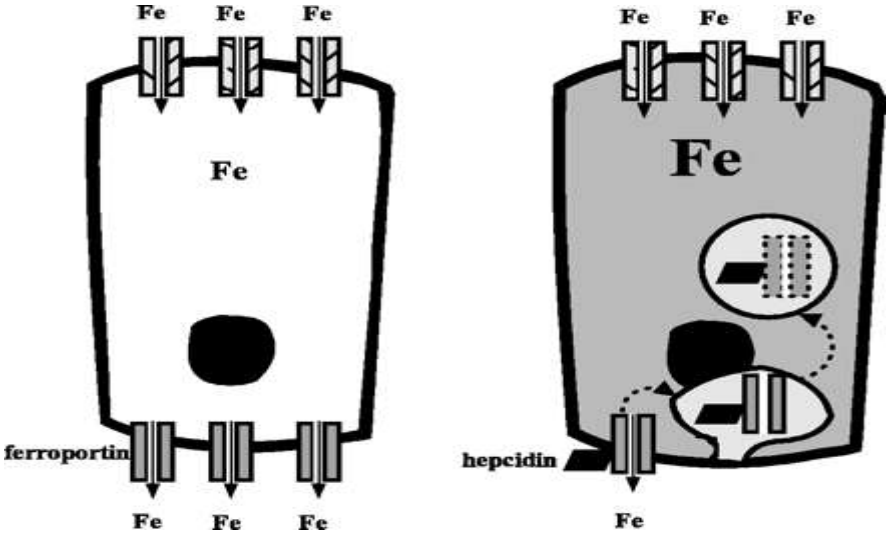

Figure (2.2): Iron transport from the enterocytes cytoplasm to plasma transferrin (Frazer et al., 2002).

Hepcidin organizes ferroportin expression on the basolateral membrane of enterocytes. As shown in Figure 2.2; *Left*: iron deficiency, with hepcidin secretion blocked and ferroportin strongly expressed on the basolateral membrane. Iron absorption is maximal. *Right*: iron excess. The liver excretes hepcidin, which interrelates with ferroportin molecules on the basolateral membrane, leading ferroportin to be endocytosed and ruined. Iron distribution from enterocytes is reduced, and the cells fill with iron. Finally, iron-filled enterocytes will be shed into the lumen of the intestine. The direct interface of hepcidin with ferroportin should not be the only path by which ferroportin thickness on cell membranes is regulated. There is evidence that ferroportin mRNA levels are also regulated by iron (Frazer et al., 2002; McKie et al., 2000).

Besides direct impacts on ferroportin and iron export, hepcidin would be likely to have secondary impacts on cellular iron intake. These indirect impacts would be motivated by increasing intracellular iron concentrations in enterocytes, macrophages, or hepatocytes that would, for example, block the synthesis of the iron-regulatory element-containing DMT1 splice variant. Ferroportin is also existed on enterocytes and macrophages. By preventing ferroportin, hepcidin stops enterocytes of the intestines from secreting iron into the hepatic portal system (Figure 2.3), which effectively reducing iron absorption. The iron that released from macrophages is also prohibited by ferroportin inhibition; therefore the hepcidin keeps iron homeostasis. Hepcidin action is also incompletely responsible for iron sequestration happened in anemia of chronic

disease and levels are raised in people with renal failure. Hepcidin has exposed justly reliable antifungal activity and this activity currently seems to be unreliable. The present scientific evidence proposes that hepcidin is an essential regulatory hormone and its main action is to regulate systemic iron homeostasis (Ashby et al., 2010).

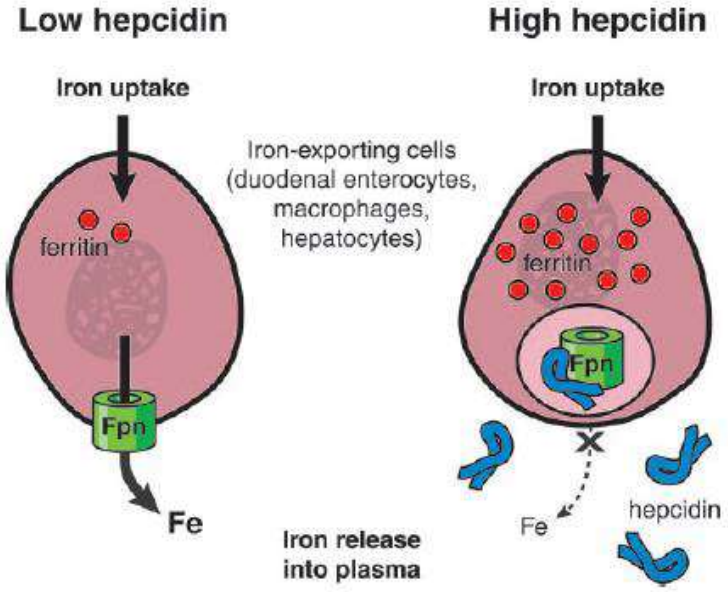

Figure (2.3): The mechanism of hepcidin-mediated cellular iron regulation (Ganz, 2007). [***Fpn***: Ferroportin channel]

2.4.6 The hepcidin-ferroportin interaction

When it's acting on ferroportin, hepcidin dominates the central inflows of iron into plasma: from duodenal enterocytes absorbing dietary iron, from macrophages implicated in the recycling of iron from senescent erythrocytes and other cells, and from hepatocytes implicated in iron storage (Figure 2.4). Throughout pregnancy, fetal hepcidin dominates the placental transfer of iron from maternal plasma to the fetal circulation. When hepcidin concentrations are low, iron moves in blood plasma at a high rate. When hepcidin concentrations are high, ferroportin is affected, and iron is stuck in macrophages, enterocytes, and hepatocytes. Plasma iron concentrations and transferrin saturation reveal the difference between the hepcidin/ferroportin-regulated transfer of iron to plasma and iron consumption by the erythropoietic BM and, to a lesser amount, other tissues. The plasma transferrin compartment is moderately small, and its iron content therefore turns over every 3 hours or so, letting iron concentrations to reply rapidly to changes in hepcidin concentrations (Delaby et al., 2005).

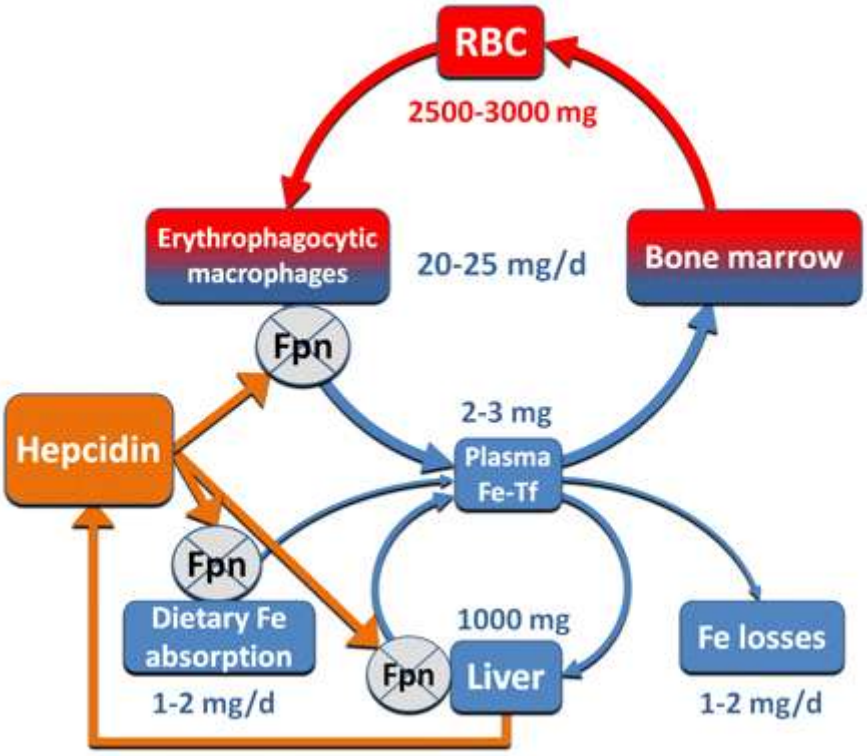

Figure (2.4): Hepcidin interaction with ferroportin controls the main iron flows into plasma (De Domenico et al., 2007).

Iron flows and reservoirs are depicted in blue, iron in hemoglobin in red, and hepcidin and its effect in orange. RBC indicates red blood cell; and Fpn, ferroportin. The role of hepcidin in regulating the absorption of dietary heme, the central form of absorbable iron in human and other carnivore diets, has not been experimentally inspected. To the amount that heme is metabolized to ferrous iron within enterocytes; its transfer to plasma would still count on ferroportin and would therefore be subject to hepcidin regulation (Delaby et al., 2005).

2.4.7 Hepcidin and inflammation

Infection and inflammation superfat hepcidin synthesis. Patients with myeloma, inflammatory bowel disease, sepsis, burns, and C reactive protein (CRP) levels >10 mg/dL show significantly raised hepcidin levels (Sharma et al., 2008). During the inflammatory process, macrophages are stimulated, and this stimulation depends on the hardness of inflammation. Activated macrophages cast a network of cytokines, among these cytokines is interleukin-6 (IL-6), which is one of the main inducers of hepcidin expression; an increase in hepcidin levels lastly results in hypoferremia. Hepcidin works

to prevent releasing of iron from macrophages as well as intestinal iron absorption. In state of inflammation, hepcidin creation is no longer regulated by iron burden (i.e., if the iron level is low, hepcidin synthesis should be down regulated), but is rather amplified through IL-6 stimulation. Serum iron was established to affect hepcidin synthesis in healthy volunteers, in whom the initial existence of hepcidin in the urine was measured after an oral iron administration dose that did not affect iron storage. Serum iron is considered as an induction indicator for hepcidin creation and impacts serum transferrin saturation percentage. In the state of inflammation, hepcidin can also be created by myeloid cells via the motivation of TRL4, a receptor placed on the membranes of neutrophils and macrophages (Peyssonnaux et al., 2006).

2.4.8 Hepcidin and anemia

Redefining the pathogenesis mechanisms of anemia has been made possible by understanding the physiological processes of hepcidin.

2.4.8.1 Hepcidin and IDA

In neat IDA, urinary and serum hepcidin attentions are suggestively reduced and are even unnoticeable by some methods presently in use. Even in the absence of anemia, hepcidin seems to be a sensitive signal of iron deficiency. Also, compared to hematocrit or hemoglobin, a reduction in hepcidin is an initial sign of iron deficiency together with transferrin saturation and reduced ferritin (Lasocki, Longrois, Montravers, and Beaumont, 2011).

2.4.8.2 Iron-refractory iron deficiency anemia

Iron-refractory iron deficiency anemia (IRIDA) is a genetically transmitted hypochromic microcytic anemia. It is featured by amplified hepcidin creation because of gene change in the suppressor matriptase-2 (TMPRSS6). Extracellular BMP2, BMP4, and BMP6 bind to the co-membrane receptor m-HJV as well as BMP receptor (BMPR). This condition activates the phosphorylation of SMAD1, SMAD5, and SMAD8 as well as the construction of heteromeric complexes with SMAD4 as the common mediator. After nuclear translocation, heteromeric SMAD complexes stimulate the transcription of the *Hamp* gene, which is responsible for hepcidin creation. Hepcidin transcription is negatively regulated by soluble HJV (s-HJV), which perform as an

antagonist of the BMP pathway, opposing with m-HJV for BMP ligands. When matriptase-2 is mutated, hepcidin growths, causing chronic inhibition of iron absorption and consequent anemia (Ramsay et al., 2009).

2.4.8.3 Anemia with iron overload

In β-thalassemia and congenital dyserythropoietic anemia, anemia is featured by iron excess. Patients who do not obtain transfusions suffer critically reduced serum and urinary hepcidin levels. Amplified erythropoietic action and the absence of hepcidin modification because of iron overload overturn the signal for the creation of hepcidin itself. In unproductive erythropoiesis syndromes, the suppression of hepcidin creation is regulated by *GDF15* and *TWSG I* (Tanno et al., 2009). Hepcidin levels are much higher in chronically transfused patients than that in non-transfused patients due to iron overload and useless erythropoiesis. In non-transfused thalassemic patients, iron is kept in hepatocytes instead of macrophages, just like transfused thalassemic patients. The outcome of this dissimilar iron cellular distribution is that serum ferritin is much lower in non-transfused patients and does not sufficiently reflect liver iron storage (Kearney et al., 2007).

2.4.8.4 Anemia of chronic disease/inflammation

Patients with chronic inflammatory disorders, infections, and cancers have "anemia of chronic disease/inflammation" (ACD). Hepcidin is high in the following inflammatory circumstances: multiple myeloma, inflammatory bowel disease, critical disorders, rheumatic diseases and chronic infections (Sharma et al., 2008). There is an infrequent form of iron-refractory hyposideremic anemia commonly extant in hepatic adenoma. The quick elimination of the adenoma resolves the hypoferremia and the consequent anemia, perhaps because the tumor is the reason of autonomous hepcidin creation. Obesity is also considered as a chronic inflammatory state that can lead to hyposideremia. In both anemia and hyposideremia, the high hepcidin level helps distinguish ACD from IDA. A condition of "mixed anemia" can happen in chronic inflammatory diseases including bleeding and/or malnutrition. Under these circumstances, the hyposideremia could respond the hepcidin increase mediated by inflammation. A true iron deficiency from non-intestinal absorption by hepcidin may grow when inflammation is extant for years (Tussing-Humphreys et al., 2009).

2.4.9 Hepcidin in the pathogenesis of iron disorders and other diseases

Hemochromatosis (HH), the most communal form of genetic iron excess, is separated into two groups: iron excess linked with imperfect or suppressed hepcidin gene and ferroportin disorders (Piperno, Mariani, Trombini and Girelli., 2009). The first group of diseases is produced by change in four genes: *HFE-1*, *HJV*, *TfR-2* and *HAMP*. Patients with imperfection of these genes have low hepcidin mRNA level in comparison with normal subjects. The change in *HAMP* reasons infrequent form of juvenile hemochromatosis (JH) type 2B and leads to down regulation of hepcidin expression (Politou and Papankikoloau, 2004). Additionally, juvenile hemochromatosis is linked with lower hepcidin level than in adult forms of hemochromatosis (Piperno et al., 2009). Hepcidin, as a severe phase protein is considered as the key mediator of anemia observed in inflammatory disorders known as anemia of chronic diseases (ACD) (Guo et al., 2009). Pathogenesis of ACD is linked with reduced iron absorption and impaired mobilization of iron stores (Figure 2.5). The individuals with the anemia of inflammation, featured by disorder of iron absorption, hypoferremia and hyperferritinemia, have advanced hepcidin levels than healthy subjects (Lee, Gelbart, Waalen and Beutler, 2008). The higher attention of serum hepcidin in patients with ACD than in the healthy people can be clarified by the IL-6 rise (Darshan and Anderson, 2009). Remarkably, the connection between the IL-6 and hepcidin level was detected in patients with severe inflammatory reaction and in healthy volunteers after lipopolysaccharide injection (Kanda et al., 2008). The amplified level of serum hepcidin is detected in many chronic inflammatory diseases such as: sickle cell disease (SCD), myelodysplasia, chronic kidney diseases, coronary artery disease (CAD) and thalassemia, glucose-6-phosphate dehydrogenase deficiency (Malyszko et al., 2006). The new studies have revealed high hepcidin level during radiotherapy for prostate cancer in individuals with severe proctitis and after hematopoietic stem cell transplantation (SCT) (Christiansen et al., 2007). The overexpression of hepcidin has been exposed in clinical studies on dysmetabolic iron overload syndrome (DIOS). In patients with DIOS, the iron absorption is significantly reduced than in controls with normal iron status (Ruivard et al., 2009). In other liver diseases like obesity related to non-hereditary mild iron overloading hepatic disease (NHIOD), alcoholic liver disorders and hepatitis C virus infection (HCV) enterocytes are resistant to circulating hepcidin,

while macrophages are more sensitive (Swinkels and Drenth,2008). Serum hepcidin associates positively with hepatic hepcidin mRNA level and ferritin level in chronic hepatitis C (CHC) (Fujita et al., 2008). Hepcidin may be a prognostic and monitoring test of iron excess in patients with NHIOD and HCV. Regularization of hepcidin attentiveness may be also a sign of HCV eradication (Swinkels and Drenth, 2008).

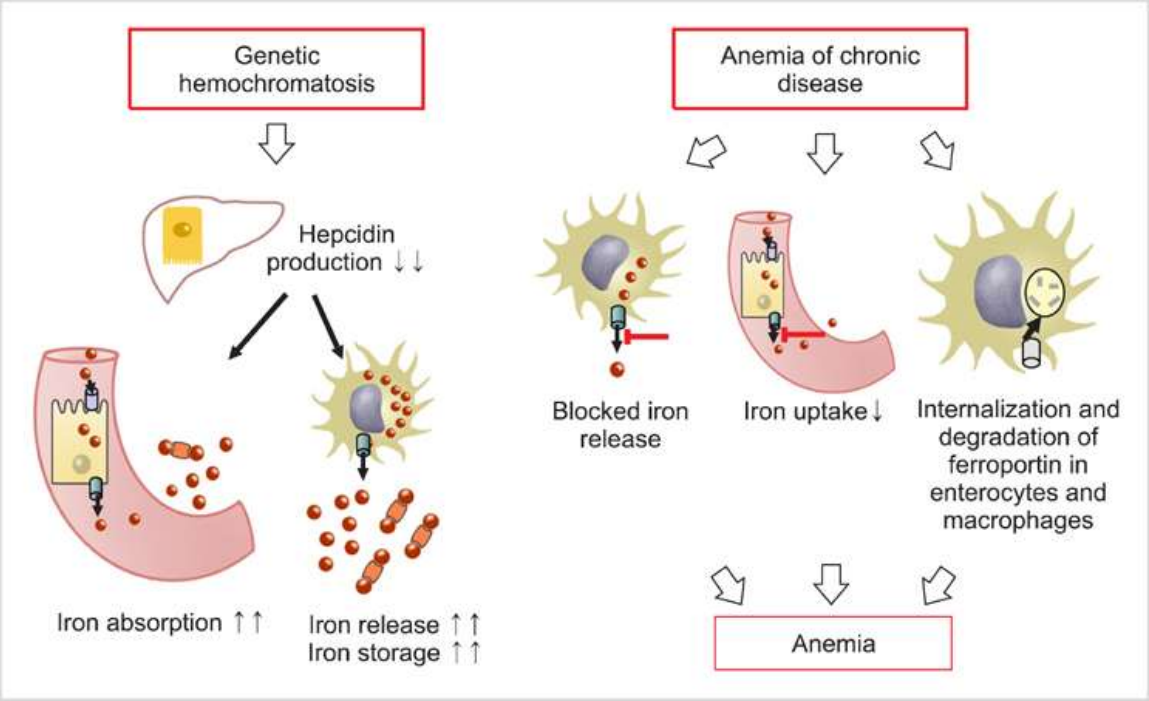

Figure (2.5): Pathophysiology of hemochromatosis and anemia of chronic disease (Guo et al., 2009).

2.4.10 Hepcidin as a potential diagnostic and therapeutic tool

The detection of hepcidin in 2000 opened the way to comprehend the iron metabolism and helped to illustrate the path mechanisms of many diseases. The studies on hepcidin raise the question of the usage of hepcidin as a diagnostic and therapeutic tool in many diseases. Hepcidin measurement can be useful test differentiating anemia of chronic diseases (ACD) from iron deficiency anemia (IDA), as it is well-known that hepcidin creation is induced by inflammation (ACD) and reduced in iron deficiency states (IDA) (Nemeth et al., 2004 and Brugnara, 2008). The utilization of hepcidin in diagnosis and monitoring of hemochromatosis is one of the utmost promises for the practical application of hepcidin assay. Moreover, the likely therapeutic value of hepcidin is examined. The advancement of synthetic hepcidin should be valuable in the treatment of hemochromatosis and other iron-loading conditions (Laftah et al., 2004). In 2008,

Ganz et al made successful validation of a competitive enzyme-linked immunoassay (C-ELISA) for human hepcidin. This humble and strong assay can be used in spotting physiologic and pathologic changes in serum or urine hepcidin levels. It is possible that this test will be extensively obtainable for use in clinical chemistry laboratories in the near future. However, there is still a lot remains to be discovered on the biology and function of hepcidin, its signaling pathways are as yet to be delineated. Additional studies are required to define exactly the hepcidin role in iron metabolism homeostasis and its usefulness in the diagnosis and treatment of iron disorders (Ganz et al., 2008).

2.5 Previous Studies

In Turkey a study involved 103 healthy Turkish primigravida women with a normal pregnancy. Blood samples were obtained at 11–14, 24–28 and 30–34 weeks of gestation. Hemoglobin, hematocrit, red cell indices, white blood cell count, platelet count, iron status indicators (plasma iron, transferrin, ferritin levels and iron binding capacity), serum hepcidin, interleukin-6 and C-reactive protein levels were analyzed. The proportions were compared using Pearson's $\chi 2$ test or Friedman's test. The mean serum hepcidin concentrations at 11–14, 24–28 and 30–34 weeks of gestation were as follows: 7.8 ± 3.4 ng/ml, 8.6 ± 3.1 ng/ml and 7.3 ± 3.0 ng/ml, respectively. The mean serum ferritin concentrations with median values at each trimester were 14.2 (11.5), 9.5 (8.8) and 11.2 (9.3), respectively. The mean serum CRP values at each trimester were 5.1 (4.0), 5.5 (4.6) and 6.0 (5.5), respectively. The serum hepcidin levels were not related to iron status or the hemoglobin, IL-6 or CRP levels. There was no association between serum hepcidin and serum ferritin, IL-6 or CRP concentrations in each trimester among low-risk pregnant women (Simavli, Derbent, Uysal, and Turhan, 2014).

Another study that was conducted on thirty-one healthy pregnant women were included and 81 blood samples from the three trimesters Hepcidin concentration decreased gradually from the first to the second and third trimester to undetectable levels (≤ 0.5 nmol/L) which was paralleled by decreasing hemoglobin levels and changes in iron parameters indicative for iron deficiency. During gestation hepcidin levels correlated with iron parameters, but not with inflammatory markers. Postpartum, hepcidin increased immediately to levels similar as assessed at early pregnancy. They conclude that hepcidin levels were suppressed during the second and third trimester of pregnancy,

which was likely determined by the occurrence of iron deficiency. These data give insight in iron homeostasis during normal pregnancy (Van Santen et al., 2013).

In Bangladesh a study consisted of a total of 190 pregnant women from northwestern Bangladesh. This was done in order to extend the range of ferritin concentrations to lower values in the sample of women, thus allowing for the elucidation of the relationship between hepcidin and iron status .It is noteworthy that women in this study were in the first trimester of pregnancy, when the demand of the body for iron is relatively low due to the cessation of menstruation. Iron requirements increase through the second and third trimesters of pregnancy to support the expansion of the red blood cell mass and tissue development of the placenta and fetus. Future work is needed to characterize changes in hepcidin in relation to iron status as pregnancy progresses and iron deficiency becomes more acute. This study provides insight into the role of hepcidin in iron deficiency associated with pregnancy. Hepcidin is likely to be a key regulator of iron metabolism during pregnancy, and this study provides strong evidence that iron status in particular influences hepcidin concentrations among pregnant women of Bangladesh (West et al., 2006).

A Bulgarian study involved 50 pregnant women where serum hepcidin levels were determined. The samples were taken in the University Hospital "Michin Dom" for a period 2013 – 2014 year. They found statistically significant differences in serum hepcidin levels between measured groups: pregnancy without anemia – 20.5 ± 6.2 μg/L; pregnancy with IDA – 1.3 ± 0.6 μg/L; Serum ferritin levels showed significant differences between two groups: pregnancy without anemia – 59.1 ± 23.6 ng/mL; pregnancy with IDA – 17.6 ± 7.1 ng/mL. Hepcidin concentration decreases gradually from the first to the second and third trimesters to undetectable levels. During pregnancy levels of hepcidin correlate with iron parameters (Manolov et al., 2015).

In an African study which involved 50 pregnant women suffering from IDA and 50 healthy pregnant women. Blood was drawn at 22 and 32 weeks gestation for the assessment of iron status. They found statistically significant in serum hepcidin levels in IDA pregnant women ($p < 0.05$) and declines in serum iron, ferritin, transferrin saturation., the results show that the concentrations of hepcidin in IDA pregnant women were lower than healthy pregnant women (Welke, 2016).

Another study was conducted to determine serum hepcidin concentrations and its association with iron status in IDA pregnant women and healthy pregnant women, 191 pregnant women were studied. Serum hepcidin was measured by C-ELISA and compared to hematological and iron indices, including ferritin, TfR, %TSat, hsCRP, and EPO. Hepcidin in IDA pregnant women was significantly lower than in the normal pregnant women. Serum hepcidin was <5 ng/ml **(**Waterman et al., 2010).

Another study was conducted to assess hepcidin concentrations in severe IDA pregnant women and to find out its correlation with other iron status parameters. This prospective observational study was carried out in 30 pregnant women with severe IDA, and 15 healthy non anemic pregnant women, infection/inflammatory conditions were excluded. Quantitative estimation of CBC, serum iron, ferritin, TIBC, and transferrin saturation (Tfsat) were done. Serum hepcidin concentrations were measured by double-antibody sandwich ELISA using a Human Hepcidin-25 kit. The serum hepcidin, iron and ferritin concentrations in IDA were significantly lower than non anemic controls ($p< 0.001$). Significant correlation was observed between hepcidin concentrations and other iron status parameters (Basu, Kumar, Srivastava, and Kumar 2016).

Chapter three

Materials and methods

Chapter Three

Materials and Methods

3.1 Study design

The present study is a case control one.

3.2 Study population

The target population comprised of a group of IDA pregnant women patients and healthy non IDA pregnant women.

3.3 Study time frame

The study started in May 2016 up to March 2017.

3.4 Sampling and sample size

The sample was 45 pregnant women with IDA (Hb<11) and 45 healthy pregnant women with Hb ≥11. The pregnant women aged (20-38) years were selected from Al-aqsa hospital in Deir al-Balah city, Health center of Deir al-Balah and AL-Remal Health center. Control healthy individuals with no history of IDA were selected from the general population that matches the case group in sex, age and residence.

3.5 Selection Criteria

3.5.1 Inclusion Criteria

- Pregnant women aged (20-38)
- Pregnant women in the third trimester of pregnancy
- Pregnant women who did not take iron therapy

3.5.2 Exclusion Criteria

- Patients who take iron therapy
- Patients with hemoglobinopathy
- Patients with a history of smoking
- Patients with liver and renal disease
- Obese patients

3.6 Ethical considerations

The necessary approval was obtained to conduct the study from Helsinki committee in the Gaza strip (Annex 1). Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. All the participants were given a full explanation about the purpose of the study and agreed to participate. An official letter of request sent from the Palestinian ministry of health to hospitals and primary health care in Gaza strip to improve the study facility (Annexes 2 & 3).

3.7 Limitations of the study

The study was conducted in only two cities of Gaza Strip and the sample size was not large. Sample collection was relatively difficult due to the objection of many patients to participate.

3.8 Data collection

3.8.1 Questionnaire Interview

A meeting interview was used for filling in the questionnaire which was designated for matching the study needs for cases and controls (Annex 4). All participants were interviewed face to face by the researcher herself. During the interview the researcher explained to the participants the unclear questions. Most questions were Yes / No questions. The questionnaire included questions on personal information (age, height, and weight), socioeconomic character (pregnant education, and family income/month) and medical history data.

3.8.2 Body Mass Index

Body mass index was calculated as the ratio of body weight in Kg/height in square meter. Patients were asked to remove heavy clothes and shoes before measurement of weight and height. Medical balance (Seca Model 762, Germany) was used for weight measurement. People with BMI=18.5-24.9 kg/m^2 were considered to have normal weight, people with BMI=25.0-29.9 kg/m^2 were classified overweight, people with BMI≥30.0 kg/m^2 were considered obese (WHO, 2012).

3.9 Specimen collection

Blood samples were collected from IDA pregnant women (case group) as well as from healthy pregnant women (control group).

3.10 Blood sampling and processing

Venous blood samples (5ml) were drawn from the participants by the researcher herself and dispensed into two tubes. About 2 ml were placed into EDTA vacutainer tube to determine Hb and RBC indices. The remaining quantity of blood was placed into the plain tube to allow blood to clot. Then serum sample was obtained by centrifugation (3000rpm/10 minutes) at room temperature to assay serum hepcidin, iron, ferritin and TIBC.

3.11 Resources and Equipment

- The following Equipment, Instruments and Reagents were used:
- ELISA reader (Mindray – china)
- Chemistry auto analyzer (Mindray – china)
- CBC auto analyzer (cell Dyn 1800, USA)
- Vortex mixer
- Refrigerator
- Centrifuge (Gemmy –Taiwan)
- Mechanical personal scale (Seca Model 762, Germany)
- Micropipettes set 1000, 200, 50 and 10µl
- Yellow and blue plastic Tips
- 5 ml vacutainer tubes
- Chemistry plastic tubes
- EDTA tubes
- Plastic racks
- 5ml disposable syringes
- 1 hepcidin reagent kit (DRG – Germany)
- 1 ferritin reagent kit (Accubind– USA)
- 1 serum iron reagent kit (Elitech Diagnostic system - France)
- 1 TIBC reagent kit (Elitech Diagnostic system - France)

3.12 Biochemical parameters and CBC analysis

For all study population hepcidin hormone, serum ferritin, serum iron, and TIBC. Transferrin and transferrin saturation were calculated and CBC was analyzed

3.12.1 Determination of serum Hepcidin Hormone

Quantitative serum hepcidin hormone was determined by ELISA (DRG diagnostics – Germany).

3.12.1.1 Principle of hepcidin hormone:

The DRG Hepcidin ELISA kit is a solid phase enzyme ELISA, based on the principle of competitive binding. The microtiter wells are coated with a monoclonal antibody directed towards the antigenic site of the bioactive Hepcidin 25 molecule. Endogenous Hepcidin of a sample competes with the added Hepcidin –biotin conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. Incubation with a streptavidin –peroxidase enzyme complex and a second wash step follows. The addition of substrate solution results in a color development which is stopped after a short incubation. The intensity of color developed is inversely proportional to the concentration of hepcidin in the specimen sample (Scamuffa et al., 2008).

3.12.1.2 Preparation of reagents for ELISA test:

Before running the test, the following were prepared:

Standards and controls

The lyophilized contents of the standard and controls vials were reconstituted with 0.5 ml deionized water and let stand for 10 minutes in minimum, and were mixed several times before use. The reconstituted reagents were kept at room temperature.

Wash solution

Deionized water was added to the 40-fold concentrated wash solution. 30ml of concentrated wash solution were diluted with 1170 ml deionized water to final volume of 1200ml. The diluted wash solution is stable for 2 weeks at room temperature.

3.12.1.3 Specimen storage and preparation

Specimens were capped and stored frozen at -20°C until analysis. Samples were thawed before analysis and they were inverted several times prior to testing.

3.12.1.4 Assay procedure for Hepcidin test

Each run included a standard curve.

1- Ten µl of sample buffer were added to each of this wells.
2- Twenty µl of each standard, control and samples with new disposable tips were disposed into appropriate wells.
3- Reaction mixture was incubated for 30 minutes at room temperature on a plate shaker at ≈ 500 rpm
4- A volume of 150 µl assay buffer and 100 µl enzyme conjugate were added to each of these wells.
5- Reaction mixture was incubated for 180 minutes at room temperature on a plate shaker at ≈ 500 rpm, and the contents of the wells were briskly shaked out.
6- The wells were rinsed 5 times with distilled water (400 µl per well).
7- The wells were stroked sharply on absorbent paper to remove residual droplets.
8- A volume of 100 µl of enzyme complex were dispensed into each well.
9- Reaction mixture was incubated for 45 minutes at room temperature.
10- The contents of the wells were briskly shaked out.
11- The wells were rinsed 5 times with distilled water (400 µl per well). The wells were stroked sharply on absorbent paper to remove residual droplets.
12- A volume 100 µl of substrate solution were added to each well.
13- Reaction mixture was incubated for 30 minutes at room temperature.
14- The enzymatic reaction was stopped by adding 100 µl of stop solution to each well.
15- The absorbance (OD) of each well was determined at 450±10 nm with a microtiter plate reader.

The wells were read within 10 minutes after adding the stop solution.

3.12.1.5 Calculation of Results for hepcidin

1. The average absorbance values were calculated for each set of standards, controls and specimen samples.
2. Semi-logarithmic graph paper was used; a standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. The mean absorbance value was used for each sample to determine the corresponding concentration from the standard curve.

Table (3.1): Reference values of Hepcidin

Population	Range(ng/ml)	Mean(ng/ml)	Median
Males and Females	0.25-47.66	16.45	13.47

(Kemna, Tjalsma, podust and Swinkels, 2007)

3.12.2 Determination of Serum Ferritin

Quantitative serum hepcidin hormone was determined by ELISA (Accubind diagnostics kit, USA).

3.12.2.1 Principle of Ferritin test

Immunoenzymometric sequential assay: The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-ferritin antibody. Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody- antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex.

Immobilized complex(IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step.Asuitalble substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration. A dose

response curve can be generated from which the antigen concentration of an unknown can be ascertained (Jandal et al., 1996).

Table (3.2): Reagents used for ferritin test.

Materials provided	
Ferritin Calibrators	Six vials of Ferritin calibrators at levels of 0(A),10(B),50(C),150(D),400(E)and 800(F)ng/ml. Store at 2-8°C
Ferritin Biotin Reagent	One (1) vial containing biotinylated monoclonal mouse IgG in buffer, Dye, and preservative. store at 2-8°C
Ferritin Enzyme Reagent	One (1)vial containing horseradish peroxidase(HRP) labeled anti-ferritin IgG in buffer,Dye,and preservative store at 2-8°C
Streptavidin coated plate-96wells	One 96-well microplate coated with streptavidin and package in an aluminum bag with a drying agent. Store at 2-8°C
Wash solution concentrate	One (1) vial containing a surfactant in buffered saline. Preservative has been added store at 2-8°C
Substrate A	One bottle containing Tetramethylbenzidine (TMB) in buffer. Store at 2-8°C
Substrate B	One bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C
Stop solution	One bottle containing a strong acid (1N HCL). Store at 2-8°C

3.12.2.2 Specimen collection and preparation for ferritin test

The blood was collected in a plain tube without additives or anticoagulants. It was allowed to clot, centrifuged and the serum was separated. Samples were stored at -20°C and analyzed within 30 days.

3.12.2.3 Preparation of reagents for ferritin

Wash Buffer

The content of wash solution was diluted to 1000ml with distilled water in a suitable storage container and stored at 2-3°C for up to 60 days.

Working Substrate Solution

The contents of the amber vial labeled solution 'A' were poured into the clear vial labeled solution 'B' and stored at 2 - 8°C.

3.12.2.4 Assay procedure for ferritin

1. 25µl of the appropriate serum reference, control or specimen was pipetted into the assigned well.
2. 100µl of the Ferritin Biotin Reagent was added to each well. All reagents were dispensed close to the bottom of the coated well.
3. The microplate was swirled gently for 20-30 seconds to mix, then covered and incubated for 30 minutes at room temperature.
4. The contents of the microplate were discarded by decantation and the plate was blotted dry with absorbent paper.
5. 350µl of wash buffer was add and then decanted, taped and blotted) aspirate. The step was repeated for two more times.
6. 100µl of the Ferritin Enzyme Conjugate was added to each well and then incubated for 30 minutes at room temperature.
7. The contents of the microplate were discarded by decantation and the plate was blotted dry with absorbent paper.
8. 300µl of wash buffer was add and then decanted, taped and blotted) aspirate. The step was repeated for two more times.
9. 100µl of working substrate solution was added to all wells and then incubated at room temperature for 15 minutes.
10. 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
11. The absorbance was read in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader. The results were read within 30 minutes of adding the stop solution.

3.12.2.5 Calculation of Results for ferritin

1. The absorbance was recorded for each of the wells.
2. The absorbance was ploted for each duplicate serum reference versus the corresponding ferritin concentration in ng/ml on linear graph paper
3. The best-fit curve was drawn through the plotted points.

4. To determine the concentration of ferritin for an unknown, the average absorbance of the duplicates for each unknown was located on the vertical axis of the graph, the intersecting point was found on the curve, and the concentration was read (in ng/ml) from the horizontal axis of the graph.

Table (3.3): Reference values of Ferritin (Nalmark, Reddy and Sawasky, 1996)

Males	16-220ng/ml
Females	10-124ng/ml
Newborn	22-220ng/ml
1-2 months	190-610ng/ml
2-5 months	50-220ng/ml
6months-16years	10-160ng/ml

3.12.3 Determination of Serum Iron

The determination of serum iron was applied by using Elitech Diagnostic Systems kit, France.

3.12.3.1 Clinical Significance of iron test

65 to 70% of total iron enters in hemoglobin composition: 20 to 25% is stored in cells. Plasma contains about 3 mg of iron bound to transferrin serum iron concentration increases in case of hemochromatosis, liver damage and iron intoxication. Decreased iron levels can be consequence of increased needs, dietary deficiency, bleeding, or an impaired absorption (gastro-intestinal disorders, malabsorption), serum iron level will always be interpreted with transferrin saturation data (Schreiber, 2003; Nutall and Klee, 2001).

3.12.3.2 Type of Method for iron

Colorimetric – Chromazurol B [End point].

3.12.3.3 Principle of iron test

Serum iron reacts with chromazurol B and catyltrimethyl ammonium bromide to form a colored complex. The intensity of the color is proportional to the iron concentration (Paris et al., 1986).

Table (3.4): Reagents used for iron test

Acetate buffer, PH 5.0	45mmol/L
Chromazurol B	0.2mmol/L
Cetylrimethyl ammonium bromide	2mmol/L
Guanidine hydrochloride	3mol/L
Standard iron	100µg/dl

3.12.3.4 Specimen and Storage for iron test

Serum collected was stored at -20 °C until analysis (Serum was free of hemolysis).

3.12.3.5 Assay procedure for iron test

The reagents were mixed manually as shown below

	Blank	**Calibration**	**Test**
Reagent R	1ml	1ml	1ml
Distilled water	50µl	-	-
Standard	-	50µl	-
Sample	-	-	50µl

Table (3.5): Reference values of Iron (Schreiber, 2003)

Newborn	100-250µg/dl
Infant	40-100 µg/dl
Child	50-120 µg/dl
Woman	50-170 µg/dl
Man	65-175 /dl

3.12.4 Determination of TIBC

The determination of TIBC was applied by using Elitech Diagnostic Systems kit, France.

3.12.4.1 Clinical Significance of TIBC

Total iron-binding capacity (TIBC) corresponds to the maximum amount of iron that Plasma proteins can bind. It is therefore an indirect way of measuring transferrin levels,

protein which transports iron in plasma. TIBC is increased in case of iron deficiencies, pregnancy or hormonal contraception. On the contrary, it may be decreased in the event of iron overload (hemochromatosis), inflammation, diseases with important protein loss (nephrotic syndrome, chronic renal deficiency), malnutrition, liver disease, malignancies, a transferrinemia (rare genetic disease). Measuring TIBC, together with iron and ferritin, is indicated in nutritional assessments and differential diagnosis of anemia's, as well as for the evaluation and the control of patients presenting a risk of iron overload (Young and Keffker, 1994).

3.12.4.2 Type of method for TIBC

Saturation / precipitation

3.12.4.3 Principle of TIBC test

First, transferrin iron binding sites are saturated by reagent R1. Then the iron in excess reacts with magnesium carbonate (reagent R2) to give an insoluble complex which is eliminated by centrifugation. Last, total iron binding capacity (TIBC) is obtained by measuring the iron concentration in the supernatant using a reagent for determination of iron in serum (Schreiber, 2003).

Table (3.6): Reagents used for TIBC test

Reagent (1) iron saturating solution	520µg/dl
Reagent (2) magnesium carbonate	One measuring spoon supplied

3.12.4.4 Assay Procedure for TIBC test

A. Saturation of Iron Binding Sites

1. In a centrifugation tube, 1 ml of reagent R1 and 0.5 ml of sample were introduced.
2. The sample was mixed, incubated for 5 minutes and then 1 level measuring spoonful was added from reagent R2.
3. The sample was incubated for 20 minutes, shacked several times during this period.
4. The sample was centrifuged at 3000 r.p.m. for 10 minutes and the supernatant was collected.

B. **TIBC Determination:**

- Iron content of the supernatant was measured colorimetrically with the Iron Chromazurol method. With the dilution 1:3 by the reagent 1 and multiply by 3 each measurement value.

Reference values of TIBC: 250-450µg/dl (Schreiber, 2003)

3.12.5 Calculation of Transferrin Saturation

Formulas which was used: [TS = (Serum Iron / Total Iron Binding Capacity) x 100%].

Reference Range: The reference range of the transferrin saturation varies by age,

Adults: 20%-50 % (McLaren et al., 1998)

3.12.6 Calculation of Transferrin

(Punnonen, Irjala, and Rajamäki, 1997)

Transferrin saturation= (Iron/Transferrin) * 71.24

Transferrin= (Iron/Transferrin Saturation) * 71.24

Reference values of Transferrin: 204-360mg/dl (Kumar, 1999).

3.12.7 Determination of Complete Blood Count (CBC).

The test was performed by using hematology autoanalyzer (CBC) [Cell-Dyn-1800], USA. A complete blood count (CBC) is a series of tests used to evaluate the composition and concentration of the cellular components of blood. It consists of the following tests: red blood cell (RBC) count, white blood cell (WBC) count, and platelet count; measurement of hemoglobin and mean red cell volume (MCV); classification of white blood cells (WBC differential); and calculation of hematocrit and red blood cell indices The hematocrit is the percentage of blood by volume that is occupied by the red cells (i.e., the packed red cell volume). Red blood cell indices are calculations derived from the red blood cell count, hemoglobin, and hematocrit that aid in the diagnosis and classification of anemia.This test measures red blood cells and white cells. The test includes: red blood cell count, hemoglobin, hematocrit, MCV, MCHC, RDW, platelet count, white blood cell count neutrophils, lymphocytes, monocytes, eosinophils and basophils.

3.13 Statistics and Data Analysis

Data were computer analyzed using SPSS/ PC (Statistical package for the Social Science). The statistical tests of significance were used depending on the nature as follows:

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (X2) was used to identify the significance of the relations, associations and interactions among various variables between case and control.
- The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as Tthe relationship between case and controls hepcidin and biochemical parameters.
- Pearson‟s correlation test was applied.
- The results in all the above mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p<0.05$)
- Percent of difference the percentage was calculated according

$$\text{Percentage difference} = \frac{(\text{mean of patient- mean of control})*100}{\text{mean of patient +mean of control/2}}$$

Chapter Four

Results

Chapter 4

Results

The present study is a case control included 90 women (45 IDA pregnant cases and 45 healthy controls pregnant). The average age of the cases was 27.3±4.8 years whereas that of controls was 27.4±4.2 years (P>0.05).

4.1 General characteristics of study population

Table 4.1 summarizes the general characteristics of the study population according to age, income, the weight of last baby (kg), height, weight and BMI. Regarding the age of the participants, there was no significant difference between the age of controls (27.4±4.2) and that of the cases (27.3±4.8) (t=0.07, P=0.944). On the other hand, the income of controls (1817.8±423.9) was higher than that of cases (656.7±177.6) (t=14.517, P=0.000). Conversely, there is a difference in the last baby's weight (kg) between controls (3.4±0.5) and cases (2.0±0.3) (t=5.130, P=0.000). The results also showed that there is no significant difference in height between controls (162.1±5.3) and cases (160.8±4.8) (t=1.237, P= 0.219). On the other hand, the results showed that the weight of controls (72.7±10.8) is not significantly different from cases (73.6±11) (t=-0.396, P=0.693). The results of BMI showed that controls (27.6±3.4) are not signicantly different from cases (28.5±4.0) (t=-1.104 P=0.273) and illustrates the general characteristics of the study population. Taking meals regularly a mong cases, 3 (6.7%), was lower compared to controls, 43 (96.6%). The decrease in taking meals regularly was statistically significant among cases compared to controls (χ^2=71.146, P=0.000). Regarding smokers among family, 11 (24.4%) of controls and 40 (88.9%) of cases reported that they have a smoker among family and this difference beween cases and controls was statistically significant (χ^2= 38.054, P=0.000). In addition, the number of controls, 42 (93.3%), who eat fruits and vegetables was higher compared to cases, 6 (13.3%) and the difference was found to be statistically significant (χ^2=57.857, P=0.000). On the other hand, rcurrent abortions in cases was higher compared to controls, >2 times was 18 (40.0%) *vs* 3 (7.6%); one time was 21 (46.7%) *vs* 8 (17.8%); no recurrent abortions was 6 (13.3%) *vs* 34 (75.6%) respectivelly, and the difference was found to be statistically significant (χ^2= 36.142, P= 0.000). In contrast, gravida was lower in cases compared to controls, ≤3 times was 34 (75.6%) *vs* 11 (24.4%); >3 times

was 24 (53.3%) *vs* 21 (46.7) respectivelly, the difference was also found to be statistically significant (χ^2= 4.849 and P=0.023).

Table (4.1): General Characterietics of the study population

General characteristics of study population			Controls (n=45)	Cases (n=45)	Statistical test	P-value
Age	mean±SD (**Range** min-max)		27.4±4.2 (18-35)	27.3±4.8 (17-38)	t = 0.07	0.944
Income (INS)			1817.8±423.9 (1500-3000)	656.7±177.6 (400-1000)	t = 14.517	0.000*
Last baby's weight (kg)			3.4±0.5 (3-4)	2.0±0.3 (1-3)	t = 5.130	0.000*
Height (cm)			162.1±5.3 (153-173)	160.8±4.8 (150-173)	t = 1.237	0.219
Weight (kg)			72.7±10.8 (55-106)	73.6±11 (52-116)	t = -.396	0.693
BMI (kg/m^2)			27.6±3.4 (21.9-37.1)	28.5±4.0 (21.6-42.6)	t = -1.104	0.273
Taking meals regularly	No. (%)	**Yes** **No**	43 (96.6) 2 (4.4)	3 (6.7) 42 (93.3)	χ2 = 71.146	0.000*
Smoker among family		**Yes** **No**	11 (24.4) 34 (75.6)	40 (88.9) 5 (11.1)	χ2 = 38.054	0.000*
Eating fruits and vegetables		**Yes** **No**	42 (93.3) 3 (6.7)	6 (13.3) 39 (86.7)	χ2 = 57.857	0.000*
Recurrent abortion		**No** **1** >2	34 (75.6) 8 (17.8) 3 (6.7)	6 (13.3) 21 (46.7) 18 (40.0)	χ2 = 36.142	0.000*
Gravida		**≤3** **>3**	34 (75.6) 11 (24.4)	24 (53.3) 21 (46.7)	χ2 = 4.849	0.023*

*p- value significant at p≤0.05

4.2 Hepcidin hormone and other iron indicators among the study population

Table 4.2 shows the comparison between cases and controls according to Hepcidin hormone and iron parameters .There are a statistically significant differences between the values of cases and those of control group for hepcidin hormone, serum Iron, TIBC, transferrin, transferrin saturation and serum ferritin ($p \leq 0.05$). The mean difference of TIBC and transferrin were higher in cases compared to controls (432.7±72 µg/dl, 342.3±59.1 µg/dl), (308.1±52.6 mg/dl, 244.5±42.5 mg/dl) respectively. On the other hand, the mean difference of serum iron, transferrin saturation, ferritin and hepcidin hormone were higher in controls compared to cases (77.7±22.9 mg/dL, 63.2±25.3 mg/dl), (23.5±8.0%, 15.6±8.0%), (15.4±14.3 ng/ml, 8.0±9.7 ng/ml) and (7.5±7.3 ng/ml, 2.6±4 ng/ml) respectively.

Table (4.2): Hepcidin and iron parameters among the study population

Parameter	Controls (n=45) mean±SD	Cases (n=45) mean±SD	% difference	t	P-value
Hepcidin (ng/ml) Range (min-max)	7.5±7.3 (1.1-41.8)	2.6±4 (0.1-19.8)	-98.2	3.975	0.000*
Serum iron(µg/dl) Range (min-max)	77.7±22.9 (42.7-174)	63.2±25.3 (33-124.8)	-20.6	2.864	0.005*
TIBC (µg/dL) Range (min-max)	342.3±59.1 (251-480)	432.7±72.0 (279-621)	23.3	-6.512	0.000*
Serum ferritin(ng/ml) Range (min-max)	15.4±14.3 (2.8-80.3)	8.0±9.7 (1.4-64)	-63.2	2.852	0.005*
Transferrin saturation (%) Range (min-max)	23.5±8.0 (9.5-43.7)	15.6±8.0 (6.2-33)	-40.4	4.703	0.000*
Transferrin(mg/dl) Range (min-max)	244.5±42.5 (179.3-345)	308.1±52.6 (199.5-442.6)	23.0	-6.311	0.000*

*P- value significant at $p \leq 0.05$ **(TIBC: Total iron binding capacity)**

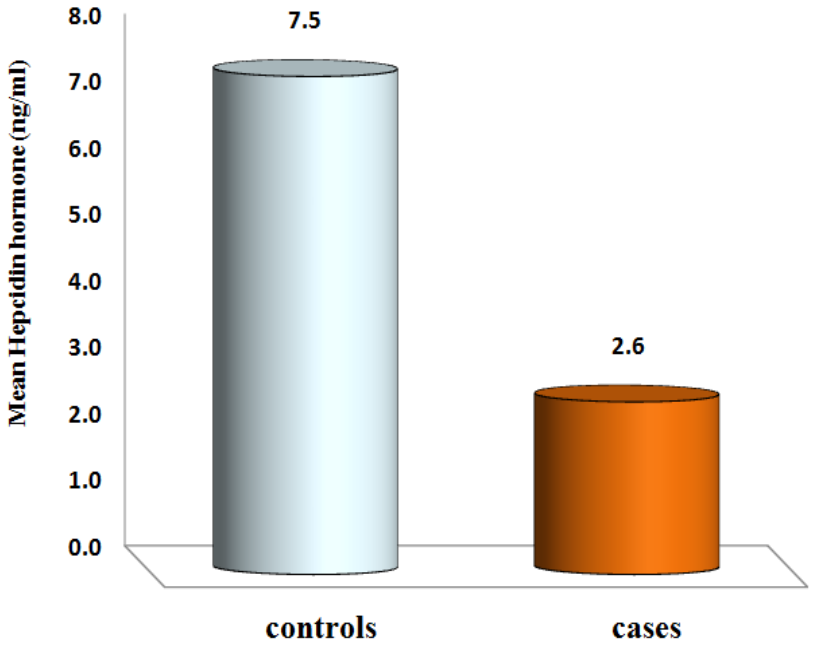

Figure (4.1): Distribution of the mean of hepcidin hormone (ng/ml) among controls and cases.

4.3 Complete blood count (CBC) indices among the study population

Table 4.3 shows the comparison between cases and control group according to CBC indices. The difference in all blood indices values between cases and controls was significant. The mean values of hepcidin hormone (7.5±7.3, 2.6±4), RBCs (4.0±0.3, 3.3±0.2), HB (11.8±0.6, 9.7±0.8), HCT (34.7±2.0, 29.4±2.3), MCV (86.3±3.3, 76.6±4.8), MCH (29.4±1.3, 25.6±2.2) and MCHC (34±0.9, 33.2±1.5) were higher in controls compared to cases. In contrast, the mean difference of RDW was higher in cases compared to controls (16.6±2.4, 13.7±0.6).

Table (4.3): Complete blood count (CBC) indices among cases and controls

CBC indices	Controls (n=45) mean±SD	cases (n=45) mean±SD	% difference	T	P-value
Hb (g/dl) Range (min-max)	11.8±0.6 (11-14.2)	9.7±0.8 (7-10.9)	-19.5	13.82	0.000*
RBC (M/UL) Range (min-max)	4.0±0.3 (3.5-5.2)	3.3±0.2 (3-3.8)	-19.2	11.138	0.000*
HCT (%) Range (min-max)	34.7±2.0 (33.0-41.7)	29.4±2.3 (22.7-32.8)	-16.5	11.847	0.000*
MCV (fl) Range (min-max)	86.3±3.3 (80-95)	76.6±4.8 (63.3-85)	-11.9	11.123	0.000*
MCH (pg) Range (min-max	29.4±1.3 (27.4-33.1)	25.6±2.2 (20.0-29.4)	-13.8	9.864	0.000*
MCHC (g/dl) Range (min-max)	34±0.9 (31.2-37)	33.2±1.5 (29.1-36.7)	-2.4	2.829	0.006*
RDW (fl) Range (min-max)	13.7±0.6 (33.0-41.7)	16.6±2.4 (22.7-32.8)	19.1	-7.7	0.000*

*P-value significant at p≤0.05. RBCs: Red blood corpuscles; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; RDW: Red cell distribution width.

4.4 Association of hepcidin according to general characteristics of the study population

Table 4.4 shows the distribution of hepcidin hormone according to general characteristics of the study population. There is a significant association between hepcidin hormone and taking meals regularly (t=3.8, P= 0.023), smoker among family (t=2.735, P=0.008), eating fruits and vegetables (t= -3.516, P= 0.020) and recurrent abortion (t= 2.639, P=0.020). Conversely, the association between Gravida (in cases and controls) and hepcidin hormone was not significant (t= -46.8, P= 0.129).

Table (4.4): Distribution of hepcidin according to general characteristics of study population

Items	**Hepcidin (ng/ml)** Mean±SD (min-max)	**% Difference**	**t**	**p-value**
Taking meals regularly				
Yes	7.4±7.3 (0.3-41.8)	-96.0	3.8	0.023*
No	2.6±4.1 (0.1-19.8)			
Smoker among family				
Yes	3.5±4.3 (0.1-19.8)	-67.9	2.735	0.008*
No	7.1±7.9 (0.2-41.8)			
Eating fruits and vegetables				
Yes	7.1±7.3 (0.5-41.8)	-89.8	-3.516	0.020*
No	2.7±4.1(0.1-19.8)			
Recurrent abortion				
Yes	3.7±4.7 (0.1-19.8)	-57.7	2.639	0.020*
No	6.7±7.7 (0.1-41.8)			
Gravida				
≤3	5.8±7.0 (0.1-41.8)	-46.8	1.532	0.129
>3	3.6±5.0 (0.1-19.8)			

*P-value significant at p≤0.05

4.5 Hepcidin correlated with iron indicators among the study population

Table 4.5 presents the correlation between hepcidin hormone levels with TIBC, serum iron, transferrin, transferrin saturation and ferritin of the study population. Hepcidin hormone showed significant negative correlation with TIBC (r= -0.551, P=0.000) and transferrin (r= -0.526, P=0.000). In contrast, hepcidin hormone showed significant positive correlation with ferritin (r= 0.558, P= 0.000), iron (r=0.547, P=0.000) and transferrin saturation (r= 0.577, P= 0.000).

Table (4.5): Correlation between hepcidin and other iron indicators among the study population

Parameters	Serum hepcidin	
	Pearson correlation (r)	P-value
Serum iron (µg/dL)	0.547	0.000*
TIBC (µg/dL)	-0.551	0.000*
Serum ferritin (ng/ml)	0.558	0.000*
Transferrin saturation (%)	0.577	0.000*
Transferrin (mg/dL)	-0.526	0.000*

*P-value significant at p≤0.05

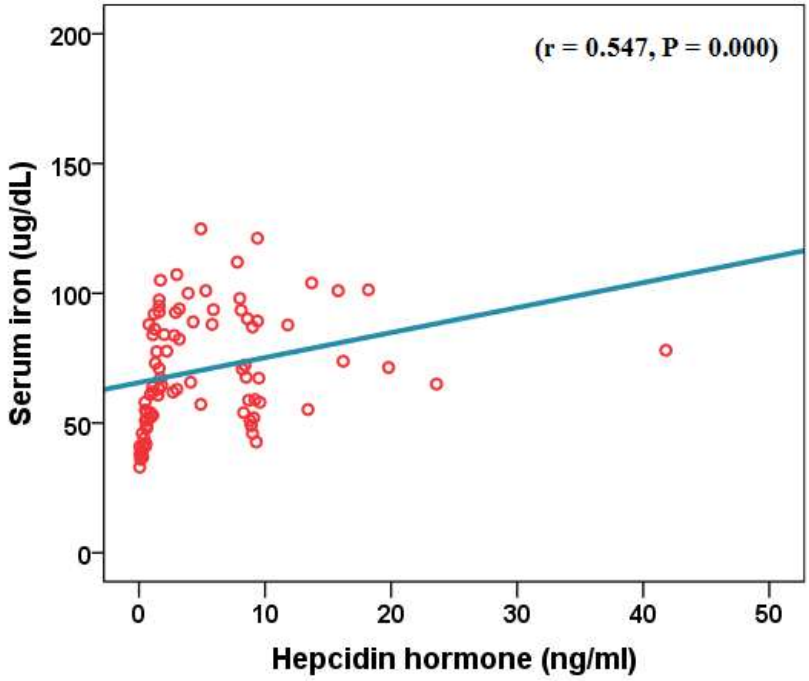

Figure (4.2): The positive correlation between hepcidin hormone and serum iron.

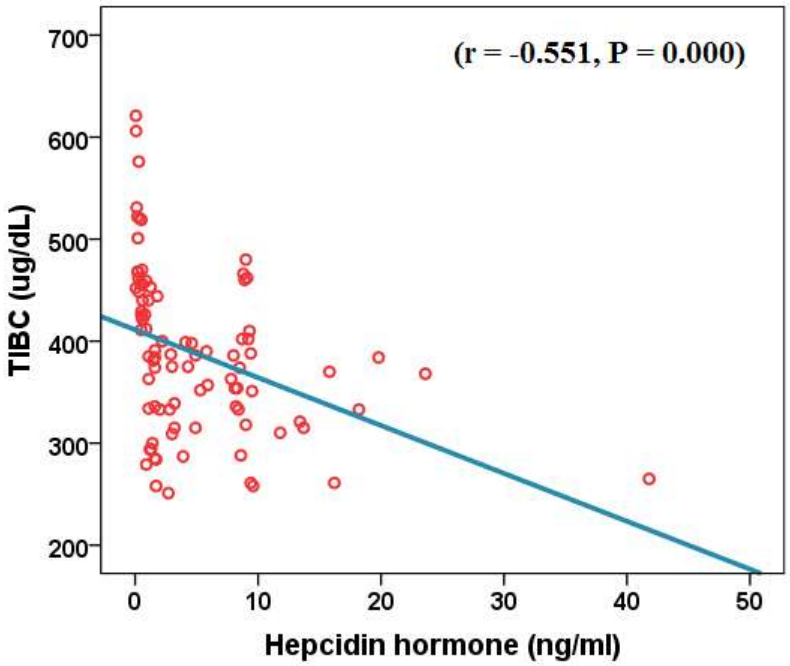

Figure (4.3): The negative correlation between hepcidin hormone and TIBC.

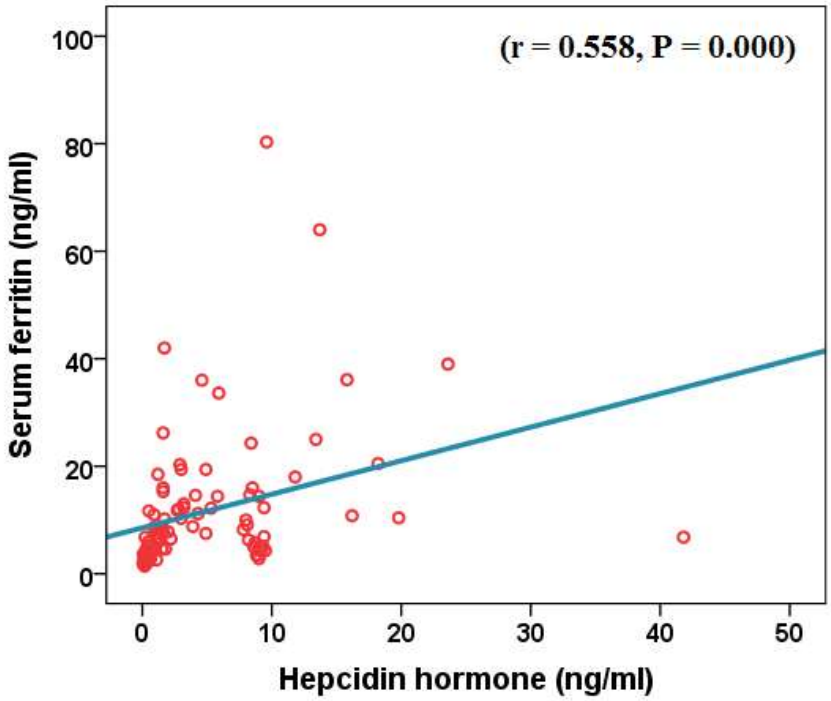

Figure (4.4): The positive correlation between hepcidin hormone and serum ferritin.

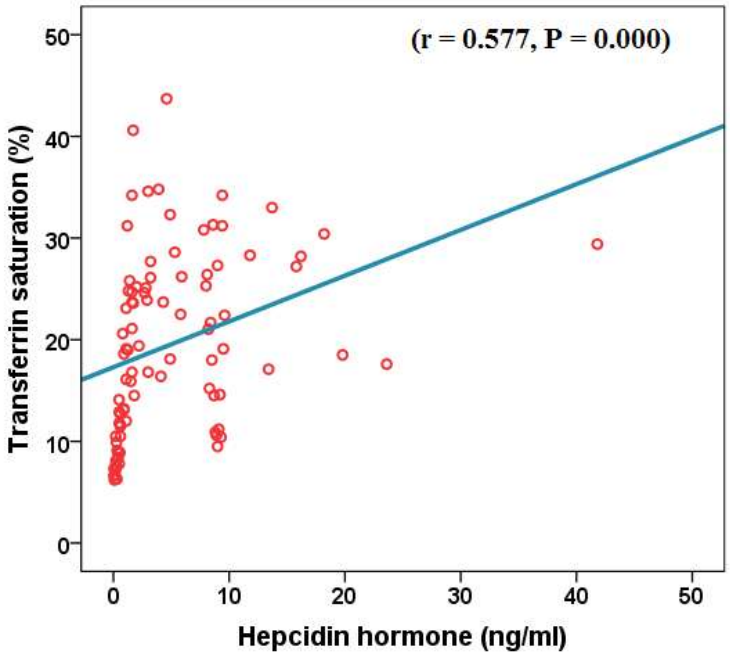

Figure (4.5): The positive correlation between hepcidin hormone and transferrin saturation.

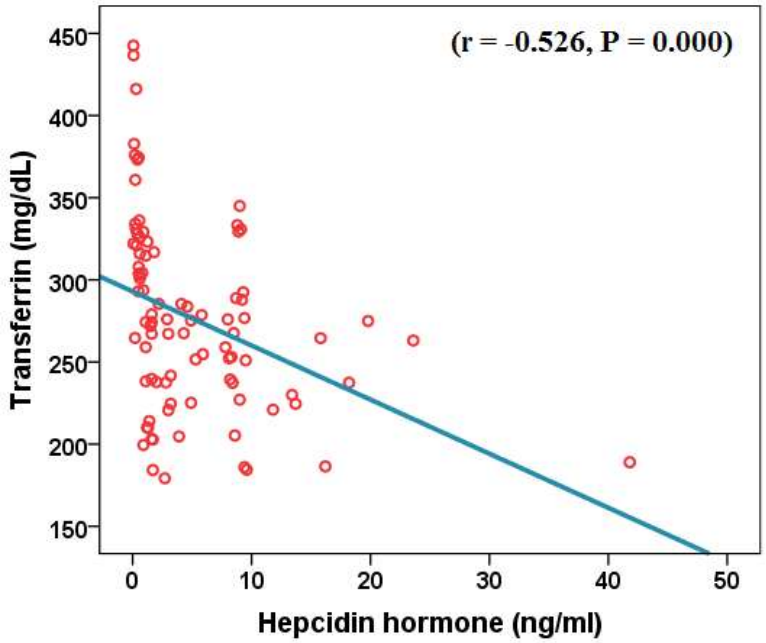

Figure (4.6): The negative correlation between hepcidin hormone and transferrin.

4.6 Hepcidin levels correlated with CBC indices among the study population.

Table 4.6 presents the relationship between hepcidin levels with Hb, R.B.Cs, HCT, MCV, MCH, MCHC and RDW. Hepcidin showed a significant positive correlation with Hb (r=0.524, P=0.000), RBCs (r=0.402, P =0.000), HCT (r=0.489, P=0.000), MCV (r=0.433, P=0.000), MCH (r=0.455, P=0.000) and MCHC (r=0.249, P=0.000). In contrast hepcidin showed a significant negative correlation with RDW (r=-0.490, P=0.000).

Table (4.6): Correlation between hepcidin and CBC indices among study population

Parameters	Serum hepcidin	
	Pearson correlation (r)	P-value
Hb (g/dl)	0.524	0.000*
RBC (M/UL)	0.402	0.000*
HCT (%)	0.489	0.000*
MCV (fl)	0.433	0.000*
MCH (pg)	0.455	0.000*
MCHC (g/dl)	0.249	0.018*
RDW (fl)	-0.490	0.000*

*P-value significant at p≤0.05

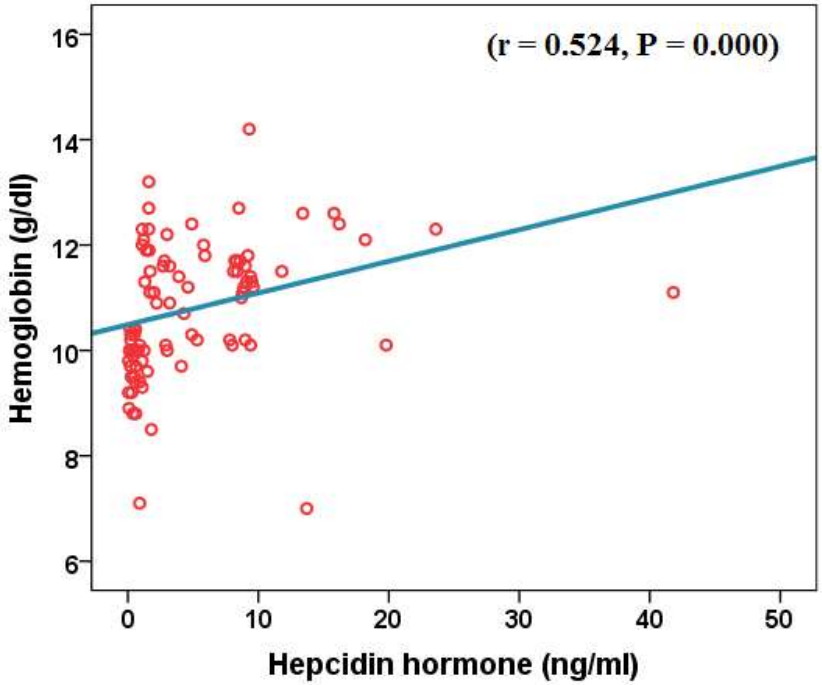

Figure (4.7): The positive correlation between hepcidin hormone and Hemoglobin.

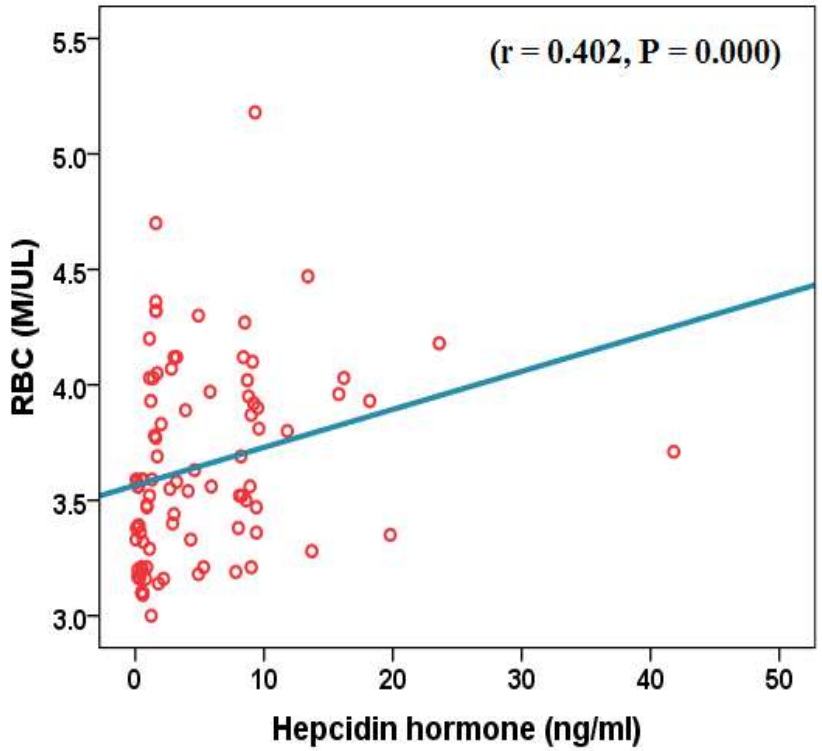

Figure (4.8): The positive correlation between hepcidin hormone and RBCs.

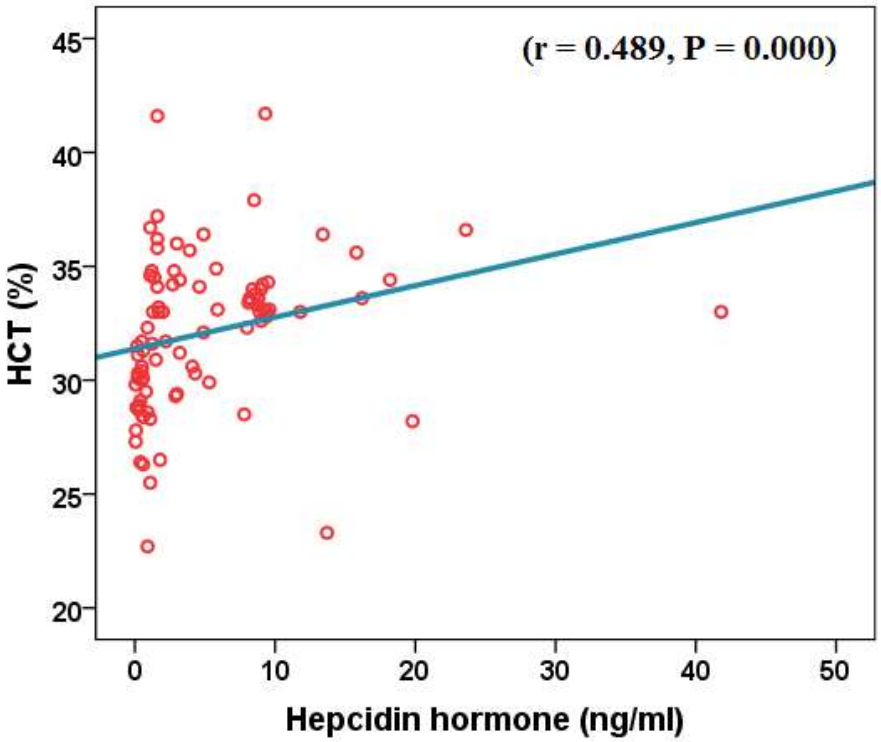

Figure (4.9): The positive correlation between hepcidin hormone and HCT.

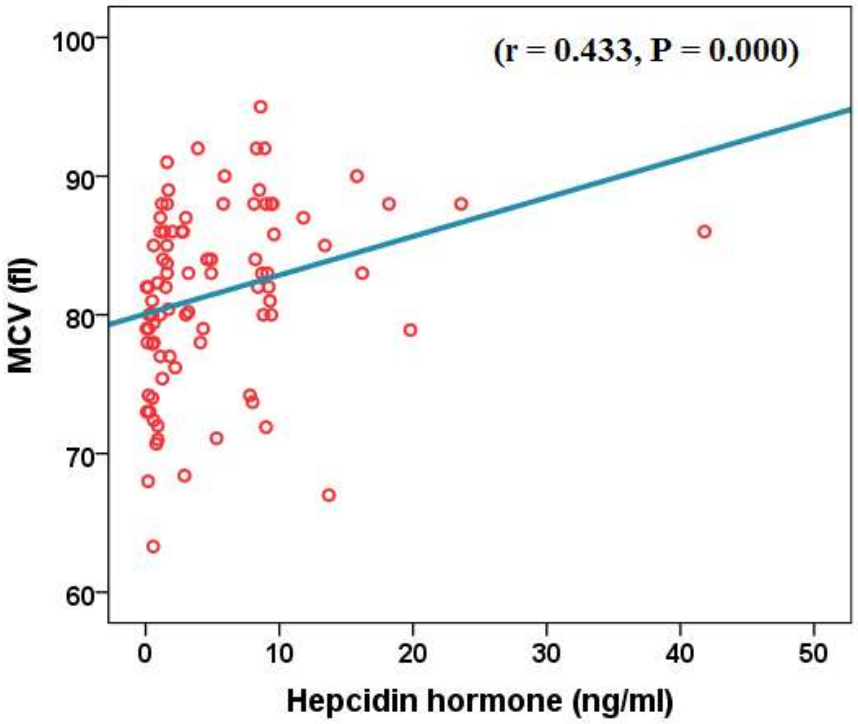

Figure (4.10): The positive correlation between hepcidin hormone and MCV.

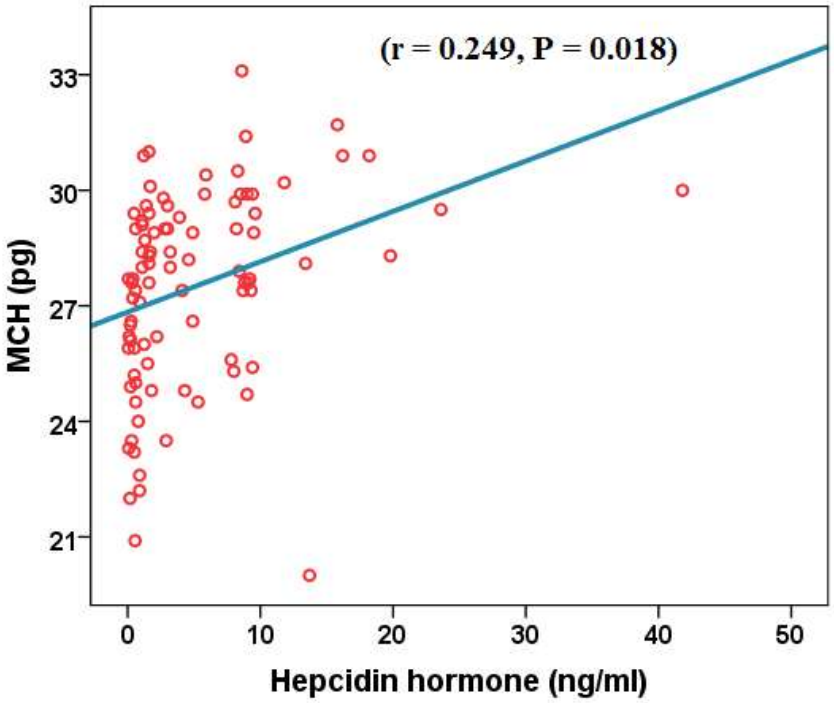

Figure (4.11): The positive correlation between hepcidin hormone and MCH.

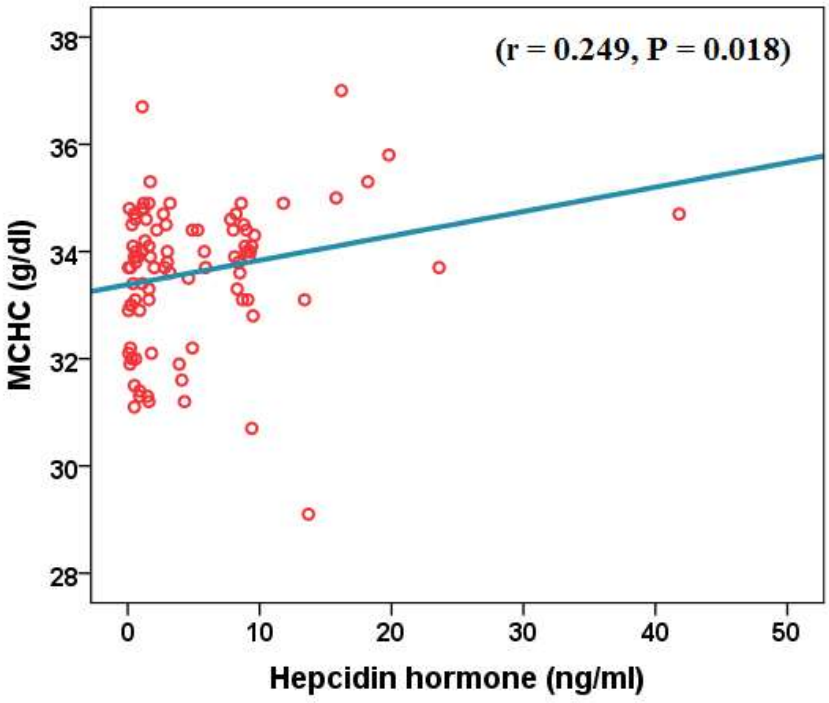

Figure (4.12): The positive correlation between hepcidin hormone and MCHC.

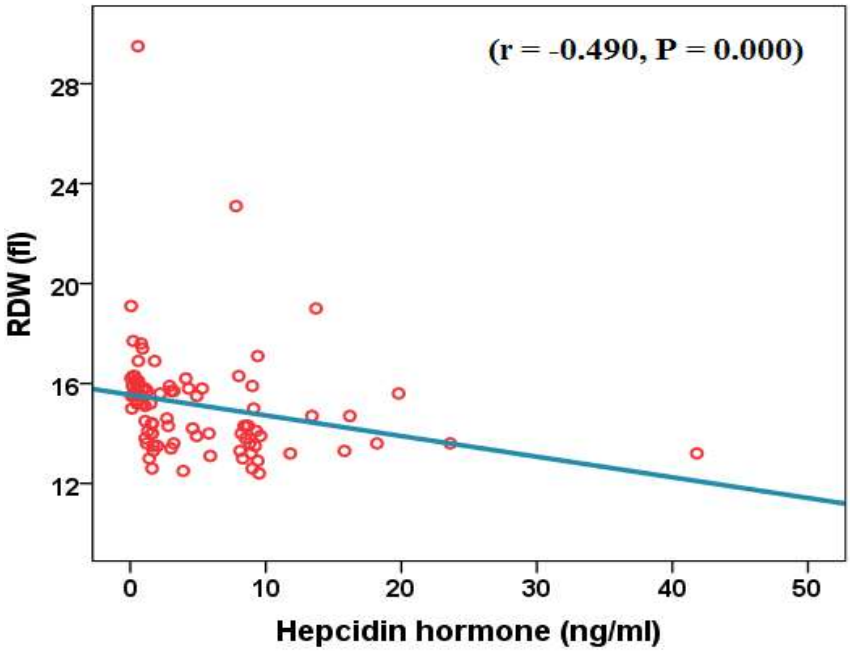

Figure (4.13): The negative correlation between hepcidin hormone and RDW.

4.7 Correlation between Hepcidin hormone levels and age, income, and anthropometric measures among the study population

Table 4.7 peresents the relationship between hepcidin levels with general characteristics. Hepcidin showed a significant positive correlation with income (r=0.513, P= 0.000). In contrast hepcidin showed a non significant negative correlation with age (r = -0.067, P=0.530), height (r =-0.026, P = 0.807), weight (r= -0.143, P =0.179) and BMI (r= -0.160, P = 0.132).

Table (4.7): Correlation between hepcidin hormone and general age, income and anthropometric measures among the study population

Parameters	Serun hepcidin	
	Pearson correlation (r)	P-value
Age (Years)	-0.067	0.530
Income (INS)	0.513	0.000*
Height (cm)	-0.026	0.807
Weight (Kg)	-0.143	0.179
Body mass index (kg/m2)	-0.160	0.132

*P-value significant at p≤0.05

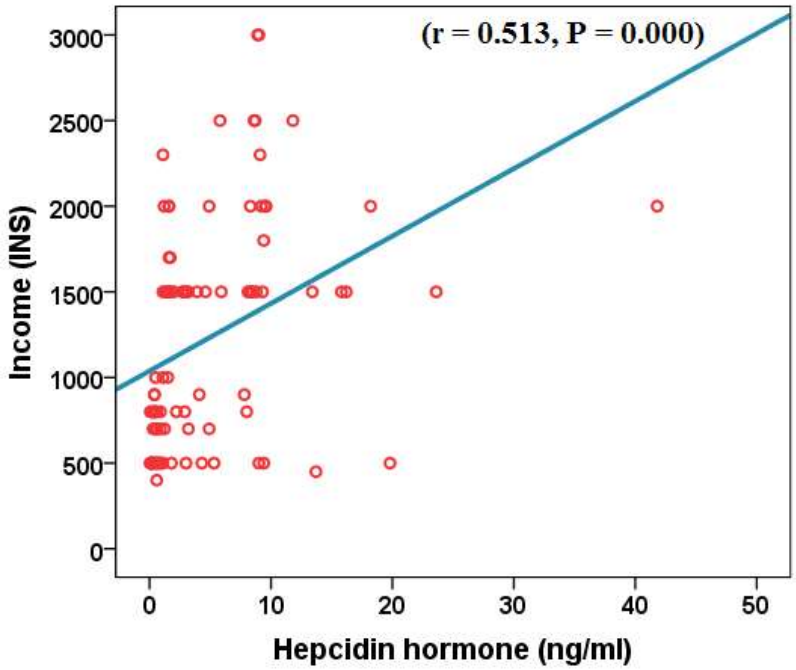

Figure (4.14): The positive correlation between hepcidin hormone and income.

4.8 Youden index cut-off points for prediction of anemic pregnant women.

In the present study, Table **4.8** illustrated the receiver operating characteristic curve (ROC curve) was detected cut off value of serum hepcidin level for diagnostic anemia among pregnant women. Serum hepcidin level was statistically significant to diagnostic anemia among pregnant women and the cut-off value for hepcidin was 1.3 pg/ml, the area under the curve (AUC) was 0.826 ($p<0.001$), sensitivity and specificity were 91.1 % & 64.4 % respectively. Positive predictive value (PPV) was 77.8 %, negative predictive value (NPV) was 71.9 %, and accuracy was 78.8% to diagnostic anemia among pregnant women (Figure **4.15**).

Table (4.8): Youden index cut-off points for prediction of anemic pregnant women

Biomarker	cases (n=45)	Controles (n=45)	Cut-off point (pg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC (95% CI)	*P* value
Hepcidin hormone	29	4	≤1.3	91.1	64.4	77.8	71.9	78.8	0.826 (0.739–0.914)	0.000*
	16	41	>1.3							

n: number of subjects; **PPV:** Positive predictive value; **NPV:** Negative predictive value; **AUC:** area under the curve; **95% CI**: 95% confidence interval; * indicates P-value significant at P≤0.05.

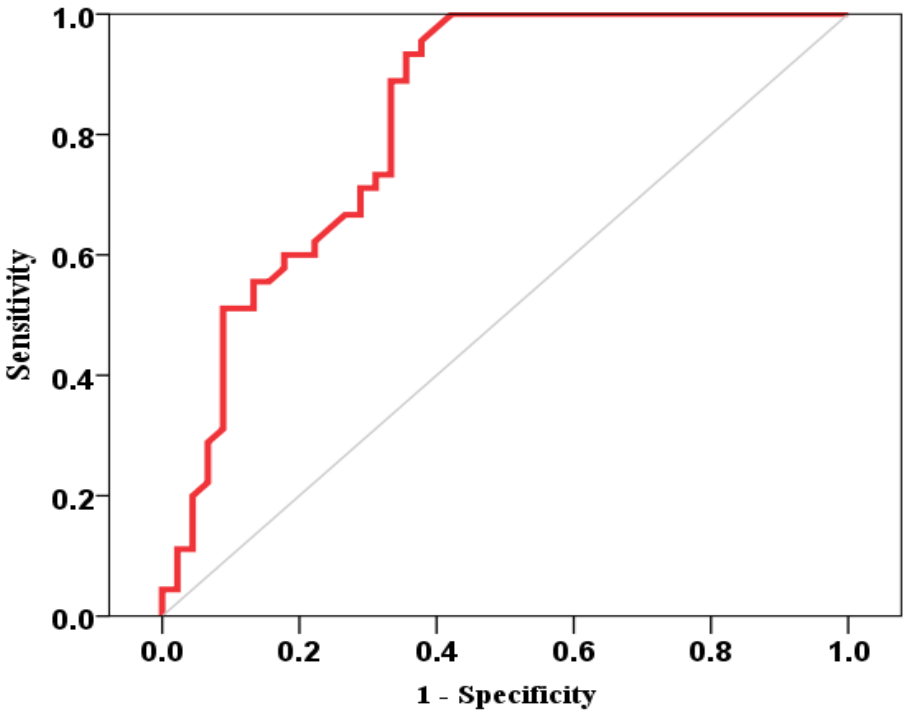

Figure (4.15): Receiver operating characteristic curve (ROC) to prediction anemic pregnant women.

Chapter Five
Discussion

Chapter five

Discussion

Iron Deficiency Anemia is a global health problem and a common medical condition seen in every day clinical practice. IDA is the most common type of anemia among pregnant women. The diagnosis and treatment of this condition can clearly be improved. The available biochemical tests of IDA are limited to routine traditional anemia factors including biochemical parameters such as iron, TIBC, transferrin, transferrin saturation, serum ferritin and CBC indices tests. On the other hand, other biochemical features in blood such as hepcidin hormone was recently linked to IDA. This discovery has revolutionized our understanding of IDA, and its measurement should advance diagnosis/treatment of this condition. The present study is the first to assess hepcidin hormone status among IDA pregnant women in Gaza strip.

5.1 General characteristics of the study population

All pregnant women who participated in this study were between 17-38 years old. They were healthy and free of any diseases, and were not on iron therapy.

5.2 Age, birth weight, and family income among the study population

The independent sample t- test showed that there is a non significant difference between cases and control groups in relation to age (p=0.944). On the other hand, the results show that there is a significant association between pregnant women with IDA and the baby's birth weight (p=0.000). Therefore, pregnant women with IDA have an increased risk for delivering a low –birth weight baby. Our results agree with those of Yip et al. (1998) who also showed that there is a significant difference between IDA pregnant women and low birth weight baby **(Yip et al., 1998).** There was a significant difference between controls and cases in terms of family income (p=0.000), where controls have higher family income compared to cases. The increase in family income means that pregnant women take good healthy foods, different types of nutrients, proteins, vitamins, minerals, and iron, which leads to maintain the body stores of iron and save them from becoming anemic.

5.3 Number of pregnancy, taking meals regularly (nutritional status) and pregnancy loss among the study population.

The present study shows that there is a statistically significance difference between cases and controls regarding the number of pregnancies (p= 0.023). Women with higher pregnancy number are more prone to have iron deficiency anema. The short interval between different pregnancies (less than one year) creates a large demand of iron, which is needed for the development of the fetus and the loss of iron during delivery increases further the iron requirment. This result is in agreement with a study performed by Hadipour et al. (2010) who found that the parity is one of the factors that could have influence of IDA (Hadipour et al., 2010). The results of the present study also show that there is a statistically significant difference between cases and controls with regard to pregnancy loss (p=0.020). IDA is one of the factors that cause fetal death and pregnancy loss (Abu-Ouf & Jan, 2015). This result disagrees with those of Scholl et al. who found that IDA is one of the factors that could have an influence on preterm delivery and don't have an influence on pregnancy losses (Scholl and Reilly, 2000). Furthermore, the results showed a statistically significant difference between cases and controls with regards to the nutritional status including taking meals regularly and eating fruits and vegetables (p=0.000). This result agrees with previous study where the researchers found that dietary status in pregnancy may be an important determinant of maternal and fetal iron status (Kumar et al., 2008).

5.4 Serum Hepcidin levels and Body Mass Index (BMI)

In the present study, serum hepcidin level was not related to BMI among the study population (p=0.132). This finding disagrees with other study that showed negative correlation between maternal BMI and hepcidin (r=-0.05, p=0.03) (Jones et al., 2016). In another study, Dao et al. (2011) found that hpcidin is significantly higher in obese pregnant women compared to non obese pregnant women (p=0.007). Serum hepcidin level was significantly higher in obese pregnant women than non obese pregnant women (p=0.02) (Dao, Sen, Aviles and Meydani, 2011).

5.5 Serum Hepcidin of the Study Population

Our results show that the mean levels of hepcidin hormone is lower in IDA pregnant women compared to controls (p=0.000). This finding is in agreement with the results of Koenig et al. (2014). Their results showed that serum hepcidin was significantly lower in IDA pregnant women and hepcidin levels decrease as pregnancy progress compared to the control group with the lowest hepcidin levels observed in the third trimester. This is due to the increase in fetal iron needs in the third trimester (Koenig et al., 2014). Another study conducted by a researcher, also agrees with our finding, showed a statistically significant difference in serum hepcidin levels between pregnant women with IDA and pregnant women without IDA (Manolov et al., 2015).

Serum hepcidin was valid tests for anemic pregnant women screening and the ability of the test to discriminate between those with anemia and those without anemia were excellent for pregnant women because the test have high value of sensitivity (91.1 %), specificity (64.4 %), PPV (77.8 %), NPV (71.9 %) and accuracy (78.8%) to diagnostic anemia among pregnant women. The present study agree with others studies, (**Pasricha et al., 2011)** were concluded serum hepcidin concentration may be a useful indicator of deficient iron stores. **(Lasocki et al., 2010)** and **(Shu et al., 2015)** shown hepcidin levels may be suppressed by iron deficiency anemia even in the case of inflammation and they concluded serum hepcidin was the most accurate threshold for iron deficiency anemia diagnosis in critically ill patients with anemia.

5.6 TIBC, Serum Iron, and Serum Ferritin of the Study Population

The results of the present study show a significant increase in TIBC concentration in IDA pregnant women compared to controls which was statistically significant (p=0.000). Furthermore, Hepcidin showed a significant negative correlation with TIBC (r=-0.551, p=0.000). Our results are compatible with those of Kwapisz et al. (2009) who showed that serum hepcidin levels were significantly negatively correlated with TIBC. All patients with IDA showed significantly lower levels of hepcidin and higher levels of TIBC compared to the control group (Kwapisz, Zekanowska and Jasiniewska, 2009). On the other hand, our results show that hepcidin has a significant positive correlation

with serum iron (r=0.547, p=0.000). The results are in agreement with those of Koenig et al. (2014) where there results showed that hepcidin was reduced in IDA pregnant women with low circulating iron (Koenig et al., 2014). In contrast, our results show that hepcidin has a significant positive correlation with ferritin (r=0.558, p=0.000) which are in compatible with those of Scholl et al. (2005) who showed that serum hepcidin levels were significantly positively correlated with ferritin (Scholl, 2005).

5.7 Transferrin and Transferrin saturation of the study population

In the present study, the mean difference of transferrin in cases was significantly higher than that in controls (308.1±52.6, 244.5±42.5 mg/dl respectively, p=0.000). While the mean difference of transferrin saturation in cases was significantly lower than that in controls (15.6±8.0, 23.5±8.0 mg/dl respectively, p=0.000). In the present study, the Pearson correlation test showed a significant negative correlation of hepcidin with transferrin (r=-0.526, p=0.000). On the other hand, hepcidin results showed a significant positive correlation with transferrin saturation (r=0.577, p=0.000). These results are in agreement with the results of Koenig et al. (2014) who showed that hepcidin is negatively correlated with transferrin and positively correlated with transferrin saturation (Koenig et al ., 2014).

5.8 Complete blood count (CBC) Indices of the study population

In the present study, the results show a significant decrease in RBCs, Hb, HCT, MCV, MCH and MCHC levels of cases compared to controls. On the other hand, RDW levels were significantly increased in cases, that means the width of the RBC was higher than those in controls. Pearson correlation test showed a positive significant correlation between hepcidin, RBC and Hb levels which are in agreement with Azab et al. (2013) (Azab and Esh, 2013). Therefore, in the present study decreased levels of hepcidin was associated with an increase in RDW in IDA patients. Another study is also in agreement with this result which found that the levels of Hb, serum iron, %saturation, ferritin and hepcidin were significantly low in IDA pregnant women than controls group (Singla et al., 1996).

Chapter six
Conclusions and Recommendations

Chapter six

Conclusions and Recommendations

6.1 Conclusions

The conclusions of the present study are:

1- The average levels of serum iron, Transferrin saturation and serum ferritin were significantly lower in cases compared to controls. In contrast, TIBC and Transferrin was higher in cases compared to controls.
2- The mean level of hepcidin was significantly lower in cases compared to controls.
3- There were negative significant associations between hepcidin levels with TIBC, and Transferrin.
4- There were positive significant associations between hepcidin levels with Iron, Ferritin, and Transferrin saturation.
5- There were negative significant association between hepcidin level and RDW, and positive significant associations between hepcidin levels with RBCs, HCT, MCV, MCH, and MCHC.
6- IDA was more prevalent among pregnant women with low income families.
7- There was a significant correlation between the IDA pregnant women and healthy pregnant women who take regular daily nutrional meals.
8- Malnutrition and parity is one of the risk factors that causes IDA.
9- Serum hepcidin level has a relationship with anemia among pregnant women. Therefore, monitoring of hepcidin levels can play an important role in management anemia among pregnant women.

6.2 Recommendations

1. Pregnant women should be a ware of the importance of regular eating and eating of iron rich foods and foods that enhance iron absorption.
2. Measurement of Hb for pregnant women on regular basis.
3. There should be educational awareness programs for anemia and iron deficiency continuously.
4. Serum hepcidin level is sensitive and specific markers of anemia among pregnant women, which provide valuable prognostic information, improve patient care, and may be used to screen anemia among pregnant women. Moreover, future investigations that use these markers in treatment pathways to improve outcomes are anticipated. Furthermore, our findings highlight the potential additive value of measuring serum hepcidin levels level in the risk assessment of anemia among pregnant women.
5. Administration of regular iron supplementation during pregnancy.
6. It is also of great importance to encourage women for early registration during pregnancy and also to attend postnatal visits during lactation for close supervision and effective follow-up.
7. Conduction of a nother study to determine the concentration of hepcidin in different gestational period.
8. Introduction of hepcidin test for IDA patients in Gaza hospital as a diagnostic tool is highly recommended.

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Annexes

Annex (1)

المجلس الفلسطيني للبحث الصحي

Palestinian Health Research Council

تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار

Developing the Palestinian health system through institutionalizing the use of information in decision making

Helsinki Committee

For Ethical Approval

Date: 01/08/2016 **Number:** PHRC/HC/131/16

Name: ESRAA M. ELNABAHEEN **الاسم:** اسراء محمود النباهين

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:

Hepcidin status among Iron Deficient Anemic Pregnant Women in Gaza strip

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/131/16 in its meeting on 01/08/2016

و قد قررت الموافقة على البحث المذكور عاليه بالرقم والتاريخ المذكوران عاليه

Signature

Member

Member

Chairman

Genral Conditions:-

1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

Specific Conditions:-

E-Mail:pal.phrc@gmail.com

Gaza - Palestine غزة - فلسطين

شارع النصر - مفترق العيون

Annex (2)

State of Palestine
Ministry of health

دولة فلسطين
وزارة الصحة

التاريخ:15/06/2016

السيد : ناصر الدين رأفت مصطفى ابوسعمان حفظه الله
مدير عام بالوزارة/الادارة العامة لتنمية القوى البشرية - /وزارة الصحة
السلام عليكم ورحمة الله وبركاته ,,,

/ الموضوع/ تسهيل مهمة باحثة/ إسراء محمد الباهس

الحاصيل //

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحثة/ إسراء محمد الباهس
الملتحقة ببرنامج ماجستير العلوم الحيانية – كلية العلوم - الجامعة الاسلامية بغزة في إجراء بحث بعنوان:

" قياس مستوى هرمون الهيبسيدين عند النساء الحوامل المصابات بانيميا نقص الحديد "

حيث الباحثة بحاجه لتعبئة استبانه وأخذ قياسات الطول والوزن وجزء من عينة دم سحبت لأغراض تشخيصية من عدد من النساء الحوامل اللاتي يعانين من انيميا نقص الحديد وعينة ضابطة ممن لا يعانين من هذه المشكلة من المراجعات لمستشفيات النساء و التوليد وعيادات الحوامل في مراكز الرعاية الأولية.

نأمل توجيهاتكم لذوي الاختصاص بضرورة الحصول على الموافقة المستنيرة من النساء الحوامل اللاتي هن على استعداد المشاركة في البحث ثم تمكين الباحثة من تطبيق ادوات الدراسة، حسب الاصول المعمول بها فيما يتعلق بهذا النوع من العينات وعلى مسئولية الباحثة، بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي و دون تحمل الوزارة أي اعباء او مسئولية.

وتفضلوا بقبول التحية والتقدير...

محمد ابراهيم محمد السرساوك
- مدير دائرة/الادارة العامة لتنمية القوى البشرية

د. محمد إبراهيم دويدار
استشاري ورئيس قسم ورمد وجراحة
Lic 6/90

المحولات

- إياد عبد الحليم توفيق العسوك(وكيل وزارة مساعد) ← نهله صبري سليمان خضر(مدير دائرة) لعمل اللازم
- ناصر الدين رأفت مصطفى ابوسعمان(مدير عام بالوزارة) ← عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة) إجراءاتكم بالخصوص
- ناصر الدين رأفت مصطفى ابوسعمان(مدير عام بالوزارة) ← فؤاد عبد الحليم توفيق العسوك(وكيل وزارة مساعد) إجراءاتكم بالخصوص
- محمد ابراهيم محمد السرساوك(مدير دائرة) ← ناصر الدين رأفت مصطفى ابوسعمان(مدير عام بالوزارة) إجراءاتكم بالخصوص
- عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة) ← عمد عودي فوزي صنهوري(رئيس قسم ادارة) للاحاطة
- عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة) ← مدحت عباس حضر حسن (مدير عام بالوزارة) لعمل اللازم
- عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة) ← كمال عواد محمد حطاب(مدير مستشفى) لعمل اللازم
- عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة) ← جمال حامد عبد الحميد المغني(مدير) لعمل اللازم

Annex (3)

State of Palestinian Ministry of health	i2	دولـة فلسطين وزارة الصحة

التاريخ: 15/06/2016

السيد : ناصرالدين رافت مصطفى ابوشعبان حفظه الله
مدير عام بالوزارة/الإدارة العامة لتنمية القوى البشرية - /وزارة الصحة
السلام عليكم ورحمة الله وبركاته ,,,

/ الموضوع/ تسهيل مهمة باحثة/ إسراء محمد الشاهين

التفاصيل //

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحثة/ **إسراء محمد الشاهين**
الملتحقة ببرنامج ماجستير العلوم الحياتية – كلية العلوم - الجامعة الإسلامية بغزة في إجراء بحث بعنوان :-

" قياس مستوى هرمون الهيبسيدين عند النساء الحوامل المصابات بأنيميا نقص الحديد "

حيث الباحثة بحاجة لتعبئة استبانة وأخذ قياسات الطول والوزن وسحب عينة دم لأغراض تشخيصية من عدد من النساء الحوامل اللاتي يعانين من أنيميا نقص الحديد وعينة ضابطة ممن لا يعانين من هذه المشكلة من المراجعات لمستشفيات النساء و التوليد وعيادات الحوامل في مراكز الرعاية الأولية

محمد ابراهيم محمد السرساوي
- مدير دائرة/الإدارة العامة لتنمية القوى البشرية

التحويلات

فؤاد عبد الحليم توفيق العيسوي(وكيل وزارة مساعد)	نهله صقر سليمان حلس(مدير دائرة)	لعمل اللازم
ناصرالدين رافت مصطفى ابوشعبان(مدير عام بالوزارة)	عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	إجراءاتكم بالخصوص
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عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	مدحت عباس خضر حسن (مدير عام بالوزارة)	لعمل اللازم
عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	كمال عواد محمد خطاب(مدير مستشفى)	لعمل اللازم
عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	جمال حامد عبد الحميد الهمص(مدير)	لعمل اللازم
عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	وليد مرزوق رزق ماضي(مدير مستشفى)	لعمل اللازم
مدحت عباس خضر حسن (مدير عام بالوزارة)	حسن محمد خليل حافظ اللوح(مدير مستشفى)	للإطلاع و توجيهاتكم بالخصوص
حسن محمد خليل حافظ اللوح(مدير مستشفى)	منذر محمد محمد عزان(طبيب مسجل مساعد / ممارس عام)	للإطلاع و توجيهاتكم بالخصوص
جمال حامد عبد الحميد الهمص(مدير)	زكريا يحي حسن الاسطل(مدير دائرة)	للإطلاع و توجيهاتكم بالخصوص
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جمال حامد عبد الحميد الهمص(مدير)	سام محمد عبدالله مسلم(مدير دائرة التمريض)	للإطلاع و توجيهاتكم بالخصوص
جمال حامد عبد الحميد الهمص(مدير)	محمد خليل محمد زقوت(مدير)	للإطلاع و توجيهاتكم بالخصوص
وليد مرزوق رزق ماضي(مدير مستشفى)	مجدي عبدالرازق عبدالله الشابا(مدير دائرة)	للإطلاع و توجيهاتكم بالخصوص

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Annex (4)

بسم الله الرحمن الرحيم

"نموذج استبيان"

نتوجة إليكم بجزيل الشكر والأمتنان لتعاونكم معنا في إنجاز بحث بعنوان:

"مستوى هرمون الهيبسدين لدى النساء الحوامل اللواتي يعانين من نقص الحديد في قطاع غزة"

1) عمرالأم الحامل ..... سنة . الطول ..... الوزن .....

2) عدد مرات الحمل ..... مرة ، وعدد مرات الإجهاض ..... مرة

3) هل تتناولين أقراص الحديد أثناء الحمل ؟؟ ☐ نعم ☐ لا

.

4) هل تتناولين الوجبات الغذائية بأنتظام ؟؟ ☐ نعم ☐ لا

.

5) ماهي نسبةهيموجلوبين الأم قبل الحمل ..... جرام/ديسيلتر

.

6) ماهو مستوى تعليم الأم ؟؟ ☐ أبتدائي ☐ إعدادي ☐ ثانوي ☐ جامعي

.

7) ماهي الفترة الزمنية بين تتابع الحمل (الولادة) ؟؟ (.....) سنة.

8) وزن الطفل عند الولادة ؟؟ (.....) كيلوجرام

9) هل يوجد عيوب وراثية عند الأطفال السابقين ؟؟ ☐ نعم ، ☐ لا .

10) ما هو معدل أوزان الأطفال السابقين ؟؟ (.....) كيلو جرام

11) هل تعانين من أي امراض ؟ ☐ نعم ☐ لا

لو نعم اذكري اسم المرض .....

12)هل يوجد مدخنين في المنزل ؟ ☐ نعم ☐ لا

13) كم معدل دخل الاسرة ؟ .....

14)هل تتناولين الفاكهة والخضار ؟ ☐ نعم ☐ لا

Questionnair

I am a researcher / Esraa M. Elnabaheen (master degree student- islamic university of Gaza) Will be very grateful if you help in completing this study which focuses on the role of hepcidin status among Iron Deficient Anemic Pregnant Women in Gaza strip.

1) Pregnant age ……. length ……. Weight…….
2) number of pregnancies ....., the number of abortions .....
3) Do you take iron tablets during pregnancy ?? ☐ Yes ☐ No
4) Do you take meals regularly ?? ☐ Yes ☐ No

5) What is the percentage of hemoglobin mothe r before pregnancy ..... g / dL.
6) What is the level of education of the mother ??

☐ Primary ☐ preparatory ☐ Secondary ☐ collectors.

7)What is the time period between pregnancies relay (birth) ??
(.....)Year.
8) The child's weight at birth ?? (.....) Kg.

9)Is there a genetic defects when former child ?? ☐ Yes ☐ No
10)What is former child weights rate ?? (.....) Kg.
11)Do you suffer from any diseases? ☐ Yes ☐ No

If yes what is the disease ……..

12) Is there any smokers at home ? ☐ Yes ☐ No
13) Howmuch the income rate of the family?.....
14) Do you take fruit and vegetables? ☐ Yes ☐ No