

National Ribat University

Faculty of Graduate Studies and Scientific Research



**Evaluation of Some Hematological Parameters
and Iron Profile among Anemic pregnant
Women in Al-Gadarif Maternity Hospital**

December 2012 – June 2014

**A thesis Submitted in Fulfilment of the Requirement
of M.Sc. Degree in Hematology**

By: Solara Ibrahim Aljack

Supervisor: Dr. Ismail Abdalrhaman

2014

الآية

قال تعالى:

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۖ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ

دُرِّيٍّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ

لِنُورِهِ مَنْ يَشَاءُ ۗ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

صدق الله العظيم

Dedication

I dedicate this effort to:

All members of my family

My parents (father and mother)

My sister (Nuha, Nahla, Nazik)

My brothers (Hytham, Hisham)

My husband (Nouraldiam)

My little kids (Rancy, Rana, Mohamed)

To all my colleagues

Acknowledgement

I thank ALLAH for all his gifts, then special thanks to Dr. Ismail Abdrahman for his close supervision. Special thanks to Dr. Abdrahman Tambal, and to medical laboratory staff at Ribat University who had dedicated their precious time to give me valuable assistance and guidance throughout the whole work. All thanks to Al-Gadarif Maternity Hospital staff.

List of Contents

| Name of Content | Page No |
|----------------------|---------|
| Verse of Holly Quran | I |
| Dedication | II |
| Acknowledgement | III |
| List of contents | IV - VI |
| List of figures | VI |
| List of tables | VII |
| English Abstract | VIII-IX |
| Arabic Abstract | X |
| Abbreviations | XI |

Chapter One: Introduction and Literature review

| | | |
|---------------|--|---|
| 1.1 | Introduction | 2 |
| 1.1.1 | Anemia | 2 |
| 1.1.1.1 | Classification of anemia | 3 |
| 1.1.1.1.1 | Morphological classification of anemia | 3 |
| 1.1.1.1.2 | Etiological classification of anemia | 4 |
| 1.1.1.1.2.1 | Increase destruction (hemolytic anemia): | 3 |
| 1.1.1.1.2.2 | Increase blood loss | 4 |
| 1.1.1.1.2.3 | Inadequate production of cells | 4 |
| 1.1.1.1.2.3.1 | Nutritional anemia | 4 |
| 1.1.1.1.2.3.2 | Bone marrow atrophy | 5 |
| 1.1.1.1.2.3.3 | Bone marrow infiltrates | 5 |
| 1.1.1.1.2.3.4 | Endocrine disease | 5 |
| 1.1.1.1.2.3.5 | Chronic renal disease | 5 |
| 1.1.1.1.2.3.6 | Chronic inflammatory disease | 5 |
| 1.1.1.1.3 | Physiological classification of anemia | 5 |
| 1.1.1.1.3.1 | Ineffective erythropoiesis | 6 |
| 1.1.1.1.3.2 | Effective erythropoiesis | 6 |
| 1.1.1.2 | Iron deficiency anemia | 7 |
| 1.1.1.2.1 | Causes of iron deficiency anemia | 7 |
| 1.1.1.2.2 | Laboratory finding | 8 |
| 1.1.1.2.3 | Iron | 8 |
| 1.1.1.2.3.1 | Iron metabolism | 8 |
| 1.1.1.2.3.2 | Body iron store | 9 |
| 1.1.1.2.3.3. | Absorbing iron from diet | 9 |

| | | |
|-----------|--|----|
| 1.1.1.3 | Clinical feature of anemia | 10 |
| 1.1.1.3.1 | Speed onset | 11 |
| 1.1.1.3.2 | Severity | 11 |
| 1.1.1.3.3 | Symptoms of anemia | 11 |
| 1.1.1.3.4 | Signs of anemia | 12 |
| 1.1.1.4 | Lab diagnosis of anemia | 12 |
| 1.1.2. | The Pregnancy | 12 |
| 1.1.2.1. | Hematological changes during pregnancy | 13 |
| 1.1.2.2 | Normal complete blood count | 14 |
| 1.1.2.3 | Hemoglobin | 14 |
| 1.1.2.4 | Hematocrite red cell indices | 14 |
| 1.1.2.5 | White blood cell count | 15 |
| 1.1.2.6 | Platelets count | 16 |
| 1.2 | Literature review | 17 |
| 1.3 | Rationale | 22 |
| 1.4 | objective | 23 |

Chapter Two: Material and methods

| | | |
|--------|--|----|
| 2.1 | Materials | 25 |
| 2.1.1 | Study design | 25 |
| 2.1.2 | Study population | 25 |
| 2.1.3 | Sample size | 25 |
| 2.1.4 | Inclusion criteria | 25 |
| 2.1.5 | Exclusive criteria | 25 |
| 2.1.6 | Ethical consideration | 25 |
| 2.2 | Methods | 26 |
| 2.2.1 | Laboratory requirement (equipment and reagents) | 26 |
| 2.2.2 | Sample collection | 26 |
| 2.2.3 | Complete blood count (CBC) | 27 |
| 2.2.4 | Principle of automated analyzer system (sysmex) | 27 |
| 2.2.5 | Preparation of thin blood film | 28 |
| 2.2.6 | Staining film | 28 |
| 2.2.7 | Examination of blood film | 28 |
| 2.2.8 | Selectra XL background and principle | 28 |
| 2.2.9 | Serum iron principle and value | 29 |
| 2.2.10 | Serum ferritin principle and value | 29 |
| 2.2.11 | Total iron binding capacity (TIBC) & Transferrin Saturation: | 29 |

| | | |
|--------|----------------------|----|
| 2.2.12 | Statistical analysis | 30 |
|--------|----------------------|----|

Chapter Three: the Results

| | | |
|--|--------|----|
| | Result | 32 |
|--|--------|----|

Chapter Four: Discussion, Conclusion and Recommendation

| | | |
|-----|----------------|----|
| 4.1 | Discussion | 45 |
| 4.2 | Conclusion | 47 |
| 4.3 | Recommendation | 48 |

References and Appendices

| | | |
|--|----------------------------|----|
| | Appendix | 50 |
| | Appendix (1) Questionnaire | 50 |
| | Appendix (2) Color Plates | 51 |
| | References | 52 |

List Of figures

| Fig No. | Title | Page No |
|-------------|---|---------|
| Figure: 3.1 | Distribution of anaemic patients by age | 32 |
| Figure: 3.2 | Average of HB and HCT among pregnant women and controls | 35 |
| Figure: 3.3 | Average of mean value platelets, WBCs and RBCs pregnant women and control | 36 |
| Figure: 3.4 | Average of MCHC, MCH and MCV among pregnant women and controls | 37 |
| Figure: 3.5 | Average of iron profile among pregnant women and controls | 38 |

List of Tables

| Table No. | Title | Page No. |
|-----------|---|----------|
| Table 3.1 | Show general characteristic Characterizes among anemic pregnant women | 33 |
| Table 3.2 | Comparison between the main value of Iron profile among pregnant women and controls | 34 |
| Table 3.3 | Comparison between mean value of CBC among pregnant women and controls | 38 |
| Table 3.4 | Comparison between average iron profile and Hb according to age group | 39 |
| Table 3.5 | Comparison between average of iron profile and HB according to level of education | 40 |
| Table 3.6 | Comparison between average of iron profile according living level | 41 |
| Table 3.7 | Comparison between Average of Iron profile and Haemoglobin according to abortion women | 41 |
| Table 3.8 | Comparison Average of Iron profile and Haemoglobin according to the number of pregnancy | 42 |
| Table 3.9 | Frequency of Morphological pattern among anaemic patients and controls | 43 |

Abstract

Background:

Anemia in pregnancy is a major public health problem in developing countries. It is associated with increased risk of maternal and perinatal morbidity and mortality. The objective of this study was to determine some hematological parameters and Iron profile among anemic pregnant women in Al-Gadarif State.

Method:

This was case control study conducted in Al-Gadarif Maternity Hospital from December 2012 to June 2014. Venous blood samples were collected in EDTA and heprin containers from two hundred (200) anemic pregnant women ($Hb < 11$) as case group and one hundred (100) non-pregnant women as a control group, the CBC and iron profile were measured using automated hematology (sysmex KX 21) and (selectra XL) chemistry analyzer. Data obtained were analyzed using the statistical package of social science (SPSS) program version 11.5.

Result

The study group had an age range between 18 to 40 years. 83.4% of pregnant women were younger than 30 years. 55.8% of pregnant women had completed their education up to the primary school, 38.7% up to the secondary school only 5.5% were University graduated.

There were statistically significant difference between case and control group in Hb (7.6 Vs. 14.4 g/dl; p value < 0.00), hematocrit (24.4% Vs. 38.8%; p value < 0.00), RBCs count (3.2×10^6 Vs. 5×10^6 ; p value < 0.00), MCV (73.4 fl Vs. 79 fl; p value < 0.00), MCH (23.7 pg/cell Vs. 27.3; p value < 0.00), MCHC (25.0 g/dl Vs. 32.0 g/dl; p value < 0.00), serum Iron 17 g/dl Vs. 114 g/dl; p value < 0.00), serum ferritin (9.4

mg/l Vs. 105 mg/l; p Value < 0.00), TIBC (428 mg/dl Vs. 285mg/dl; p value < 0.00), platelet (159×10^9 /l Vs. 175×10^9 /l ; p value 0.025). WBC can't didn't show statistically significant difference. Hb level was significantly associated with living level (p . value 0.018). Education and number of abortion and pregnancies were not associated with Hb level.

Conclusion

Some hematological parameters and Iron profile were decreased in our study. A comprehensive research in our country is needed on how to improve existing Iron supplement programs and overall health care and nutritional status of women in their reproductive years.

مستخلص البحث

فقر الدم أثناء الحمل مشكلة اساسية في البلدان النامية ، ويرتبط مع زيادة الإعتلال وفترة ما قبل الولادة الهدف من هذا العمل الحالي هو الدراسة في إنتشار نقص الحديد لدى النساء الحوامل في مدينة القضارف .

أجريت هذه الدراسة الوصفية المقطعية في مستشفى القضارف للولادة خلال أكتوبر 2012 إلى أكتوبر 2014م لتحديد معايير أمراض الدم الحديد لدى النساء الحوامل بفقر الدم ، ثم جمع ثلاث مل من عينة دم وريدي تحت ظروف معقمة مانع تجلط EDTA ومانع تجلط HEPRIIN من ثلاثمائة من النساء الحوامل منهم مائتي (200) من النساء الحوامل كان (الهيموغلوبين > 11) كمجموعة الأختبار ومائة من النساء الإصحاء وذلك لفحص الدم الشامل بواسطة جهاز (SYSMEX) وجهاز التحليل الكيميائي الآلي (SELECTRA XL) على التالي وتم تحليل النتائج برنامج الحزم الإحصائية للمجتمع (الإصدار 11.0) ومن ثم تم وضع افلام دقيقة وصبغها لرؤية شكل الخلايا ، وأظهرت نتجة متوسط خصاب الدم 7,61غ/لتر) بينما كان متوسط الخلايا المتراصه 24,41% وكان متوسط عدد كريات الدم الحمراء 1063,21 خليه/للمتر المكعب) وكان متوسط حجم الخلايا الحمراء (73,4 ف.ل) وكان متوسط نسبة الحديد (17 جرام/ ديسلتر) مايكرو غرام ، ليتر ومتوسط الفرتين (9.4 MG/L) مايكروغرام/ليتر ومتوسط الحديد الكلي (428g/dl) وأظهرت الدراسة أن خطاب الدم متوسط الخلايا المتراصه ومتوسط عدد الكريات الحمراء يقل ويحدث إختلاف كثير وأظهرت الدراسة أن هنا إختلاف كبير في مستوي الحديد والفريتين والحديد الكلي في النساء الحوامل ، ولم يتم العثور على علاقة بين الخصاب الأحمر والعمر والتعليم والإجهاض وعدد الحمل .

القيم المسبقة تظهر إنتشاء انيما نقص الحديد في مدينة القضارف وهناك حاجة الي بحوث شاملة في بلادنا حول كيفية تحسين الرعاية الصحية الشاملة والتحسين التغذية للنساء عند البلوغ .

Abbreviations

| | |
|-------|--------------------------------------|
| CBC | Complete Blood Count |
| Cumm | Cubic meter |
| DW | Distilled Water |
| EDTA | Ethylene Diamine Tetra – Acetic acid |
| FL | Femto liter |
| Hb | Hemoglobin |
| ID | Iron Deficiency |
| IDA | Iron Deficiency Anemia |
| LDH | Lactic Acid dehydrogenase |
| MCH | Mean cell hemoglobin |
| MCHC | Mean cell hemoglobin concentration |
| MCV | Mean cell volume |
| Nm | Nano meter |
| PBP | Peripheral Blood Picture |
| Pg | Pico gram |
| RBCs | Red blood cells |
| TIBC | Total Iron binding capacity |
| TWBCs | Total white blood cell count |
| WHO | World Health organization |

Chapter One

Introduction and Literature

Review

1.1 Introduction:

1.1.1 Anemia:

Anemia in pregnancy is the most common health problem in the world. Although some studies have shown a decline in the prevalence of anemia in the past three decades, anemia is still the most common nutritional problem due to iron deficiency and foliate or Vitamin B12 deficiency in infancy, childhood, and pregnancy. According to WHO data, anemia is most frequent in South Asia and Africa with the highest prevalence in West Africa. Anemia is defined as a reduction in the hemoglobin concentration of the blood. Although normal value can vary between laboratories typical values would be less than 13.5g/dl in adult males and less than 11.5g/dl in adult females. From the age of 3 months to puberty less than 11g/dl indicate anemia. As newborn infants have high hemoglobin level, 15g/dl is taken as the lower limit at birth. Reduction of hemoglobin is usually accompanied by a fall in red cell count and packed cell volume (PCV), but these may be normal in some patients with subnormal hemoglobin levels (and therefore by definition anemic). Alterations in total circulating plasma volume as well as of total circulating hemoglobin mass determine the hemoglobin concentration. Reduction in plasma volume (as in dehydration) may mask anemia or even cause (pseudo) polycythemia, an increase in plasma volume (as with splenomegaly or pregnancy) may cause anemia even with a normal total circulating cell and hemoglobin mass. After acute major blood loss, anemia is not immediately apparent because the total blood volume is reduced. It takes up to a day for the plasma volume to be replaced and so for the degree of anemia to become apparent. Regeneration of the hemoglobin mass takes substantially longer (1). Anemia in pregnancy is generally defined as hemoglobin less than 11g/dl, less than 11.5g/dl in some clinical practice guidelines

with slight variation according to the trimester of pregnancy. However hemoglobin level less than 10g/dl indicates anemia at any stage during pregnancy (2).

1.1.1.1 Classification of anemia:

1.1.1.1.1 Morphological classification of anemia:

The morphological classification is based on a correlation between red cell indices and the underlying cause of anemia. The most important measurements are red cell size (mean cell volume MCV) and red cell hemoglobin concentration (mean cell hemoglobin (MCH) or mean cell hemoglobin concentration (MCHC)(3).

Anemia with raised, normal and reduced red cell size (MCV), are termed macrocytic, normocytic and microcytic, respectively. Anemia associated with a reduced hemoglobin concentration within red cell are termed hypochromic and those with a normal MCH are termed normochromic. Characteristic combinations are of microcytosis and hypochromia, and normocytosis and normochromia. This terminology is helpful in narrowing the differential diagnosis of anemia. It is perhaps least helpful in normocytic anemia as the possible causes are numerous and diverse (3).

The value of the blood film in diagnosis, should not be underestimated. For instance combined iron deficiency (a cause of microcytosis) and folate deficiency (a cause of macrocytosis) may cause an anemia with a normal MCV. However inspection of the film will reveal a dual population of microcytic, hypochromic and macrocytic red cell (3).

1.1.1.1.2 Etiological classification of anemia:

It is less immediately helpful than the morphological classification in forming a differential diagnosis but it does illuminate the pathogenesis of anemia. The fundamental division is between excessive loss or destruction of mature red cell, and inadequate production of red cells by the marrow (3).

1.1.1.1.2.1 Increase destruction (hemolytic anemia):

Intracellular defect: In these disorder red cell exhibit at shortened life span because of intrinsic defect in the cell itself. These include the following types of anemia: Hereditary spherocytosis, glucose 6 phosphatase enzyme deficiency anemia, sickle cell anemia and thalassemia.

Extracellular defect: Destroyed because of external factors causing hemolytic these factors include parasitic infection (malaria), antibodies, chemicals and physical agents (3).

1.1.1.1.2.2 Increase blood loss:

In these types of anemia, both the speed and degree of blood loss is reflected in laboratory result: anemia resulting from hemorrhage is divided into anemia caused by acute loss and anemia caused by chronic loss (3).

1.1.1.1.2.3 Inadequate production of cells:

This category includes several entities including nutritional anemia, bone marrow aplasia, bone marrow infiltration, endocrine disease, chronic renal disease and chronic inflammatory disease.

1.1.1.1.2.3.1 Nutritional anemia:

In this group of anemia, essential substances required for erythropoiesis are either reduced or lacking. These include iron, vitamin B12, folic acid and others(3).

1.1.1.1.2.3.2 Bone marrow aplasia:

In this situation the bone marrow reduces production of red cell precursors. This aplasia can be directed either only at erythrocytic stem cells (pure red cell aplasia) or at the total hematopoietic system (aplastic anemia) in which case all formed cellular elements are quantitatively reduced. Aplastic anemia is either primary or secondary idiopathic to the action of chemical or physical agents, drugs and viral infections (3).

1.1.1.1.2.3.3 Bone marrow infiltration:

This form of anemia consists of under production that results from crowding out of erythropoietic tissue in the bone marrow by neoplastic cells (tumor cells) such as leukemia, myelofibrosis, lymphoma and myeloma (3).

1.1.1.1.2.3.4 Endocrine disease:

Anemia is a common finding of disorders affecting the thyroid, adrenal and pituitary glands (3).

1.1.1.1.2.3.5 Chronic renal disease:

This anemia is a consequence of reduced erythropoietin production resulting renal failure (3).

1.1.1.1.2.3.6 Chronic inflammatory disease:

These include infection and coeliac disease (3).

1.1.1.1.3 Physiological classification of anemia:

This physiological classification system is based on ability of the bone marrow to respond to anemia with increased erythropoietin, it involves assessing erythrocyte production using the reticulocyte count (either proportional (%) or absolute and calculated reticulocyte production index (RPI). When anemia occurs and the bone marrow is capable of responding increased numbers of young non-nucleated red cell

enter the circulation. These young polychromatophilic red cells, released prematurely from the marrow because of erythropoietin stimulation are called (shift reticulocytes) a term reflecting their premature shift from the bone marrow to peripheral blood. Reticulocytes may be increased in the circulation without an actual increased in marrow red cell production (4). To use reticulocyte count as an index of marrow red cell production effectiveness, it must be converted to the absolute number of circulation reticulocytes the RPI compensates for reticulocytes being shifted out of the marrow early and spending a longer time in the circulation (4).

1.1.1.1.3.1 Ineffective erythropoiesis:

Ineffective erythropoiesis (RPI lower than 2.0) is caused by a defective bone marrow either from intrinsic disease, lack of essential hematopoietic factors or failure in the erythropoietic mechanism itself. There are two groups which demonstrate ineffective erythropoiesis: hypo proliferative anemias and anemia secondary to maturation disorders (4).

1.1.1.1.3.2 Effective erythropoiesis:

In the case of effective erythropoiesis (RPI higher than 2.0) the bone marrow hematopoietic mechanism is functional, and factors necessary for red cell production are available. The two groups of anemia usually demonstrating effective erythropoiesis are hemolytic anemias and anemia of acute blood loss. A third for less group common of anemia's demonstrating effective erythropoiesis is that associated with low oxygen affinity. Decreased oxygen affinity hemoglobines cause increased release of oxygen to the tissue, and therefore, may cause decreased red cell number and anemia (4).

1.1.1.2 Iron deficiency anemia:

Iron is essential component in the synthesis of hemoglobin, myoglobin and several heme and metalloflavoprotein enzyme. Iron deficiency is leading cause of microcytic anemia in children and adult. When iron supply to erythroid marrow is deficient, red blood cell production is impaired and new cell released into circulation are poorly hemoglobinized. The severity of anemia and the degree of microcythosis and hypochromia generally reflect the severity and chronicity of the iron-deficiency state. The prevalence of iron deficiency in a population depend on the interaction of several factors, including adequacy of dietary iron supply and the incidence of disease states accompanied by malabsorption or chronic blood loss. In developing countries, inadequate nutrition is a major factor and iron deficiency is the principle cause of nutritional anemia. In Europe and United states chronic blood loss is more frequently responsible for the iron deficiency (5).

1.1.1.2.1 Causes of iron deficiency anemia:

The likely causes vary with the age, sex and geographic location of the patient. Iron deficiency is usually caused by long term blood loss, most often gastrointestinal or uterine bleeding and less commonly bleeding in the urinary tract or elsewhere. Particularly in elderly patients, iron deficiency may be presenting in the future of gastrointestinal malignancy. Hookworm infection is the commonest cause of iron deficiency worldwide. Malabsorption and increased demand for iron as in pregnancy are other possible causes. Poor diet may exacerbate iron deficiency but is rarely the sole cause outside the growth spurts of infancy and teenage years (3).

1.1.1.2.2 Laboratory finding:

Anemia of iron deficiency is classically microcytic (decreased MCV) and hypochromic (increased central pallor in red blood cells (RBCs)). However, in early iron deficiency, the MCV will be normal. Occasional microcytic and hypochromic RBCs may be present on the blood smear. In iron deficiency, bone marrow iron stores will be completely depleted before the hemoglobin begins to drop. The hemoglobin begins to fall before MCV begins to decrease. The reverse occurs in megaloblastic anemia: the MCV begins to increase before the hemoglobin begins to fall. MCV may be normal in combined nutritional deficiency (deficiency of iron plus either cobalamin or folic acid). However, on the blood smear hypersegmented neutrophils and possibly a dimorphic smear with both microcytes and macrocytes can be seen. Further tests are helpful in confirming the diagnosis and excluding other causes of hypochromic microcytic anemia. Measurement of serum ferritin is probably the most useful of these tests: a low level always indicates iron deficiency but a normal level does not guarantee normal stores as ferritin is increased in chronic inflammation and liver disease (in occasional difficult cases (e.g. where the patient has been recently transfused, a bone marrow aspirate is helpful in showing absence of iron stores) (3).

1.1.1.2.3 Iron:

1.1.1.2.3.1 Iron metabolism:

The balance of iron metabolism in healthy individuals predominantly reflects three variables: nutritional intake, iron loss, and current demand. The nutritional iron intake relates to the amount of digested iron in the food and the ability to absorb iron from the digestive tract. The amount of iron absorbed depends largely on the presence or absence of pathology of the gastrointestinal tract or a comorbidity (such as chronic inflammatory disease) that may result in expression of iron regulatory proteins and peptide called hepcidin, which ultimately blocks iron absorption. The

main source of iron in humans comes from the destruction of erythrocytes by macrophages of the reticuloendothelial system including the spleen or in the other words, a recycled internal iron supply (2).

1.1.1.2.3.2 Body iron stores:

The amount of storage iron has been estimated to be about 1000-2000 mg in the healthy adult male and less in the female. Storage iron occurs in two forms – ferritin and hemosiderin. Ferritin is normally predominant. In the normal person storage iron is divided equally between the reticulo endothelial cells (mainly in spleen, liver and bone marrow) hepatic parenchymal cells and skeletal muscle. Hemosiderin, the main storage form in reticulo endothelial cells, is more stable and less readily mobilized for hemoglobin formation than ferritin, which predominates in hepatocytes. In states of iron over load, haemosidern increased to a greater degree than ferritin and becomes the dominant storage form (6).

1.1.1.2.3.3. Absorbing iron from the diet:

Organic dietary iron is partly absorbed as haem and partly as inorganic iron. Absorption occur through the duodenum and is favored by factor such as acid and reducing agents that keep iron in the gut lumen in the Fe^{2+} rather than Fe^{3+} state. The protein DMT-1 (divalent metal transporter) is involved in transfer of iron from the lumen of the gut across the enterocyte microvilli. Ferro-portin at the basolateral surface controls exit of iron from the cell into portal plasma. The amount of iron absorbed is regulated according to the body's needs by changing the levels of DMT-1 and probably of ferroportin according to iron status of the duodenal villous crypt enterocyte. Iron is incorporated into the crypt enterocyte from plasma transferrin which binds to transferrin receptor in association with the protein HFE at the basal surface of the cell. In the iron deficiency less iron is delivered to the crypt cell from transferrin which is largely unsaturated with iron. The consequent iron deficiency in

the crypt cell result in increase expression of DMT-1. This occurs by the same mechanism (IRP/IRE binding) by which transferrin receptor is increased in iron deficiency. The increase expression of DMT-1 results, when the enterocyte reaches the apical absorptive surface of the duodenal villous 24 – 48h later, in increase transfer of iron from the gut lumen into the enterocyte. Increased ferroportin in iron deficiency has not yet been shown but as the m RNA has an IRE, like that of DMT-1,3 from the coding portion, it is likely that ferroportin levels also rise in iron deficiency. This would result in increased transfer of iron from the enterocyte to portal blood (1).

1.1.1.3 Clinical features of anemia:

The major adaptations to anemia are in the cardiovascular system (with increased stroke volume and tachycardia) and in the O₂ dissociation curve. In some patients with quite severe anemia there may be no symptoms or signs, whereas other with mild anemia may be severely incapacitated. The presence or absence of clinical features can be considered under four major headings (1).

1.1.1.3.1 Speed of onset:

Rapidly progressive anemia causes more symptoms than anemia of slow onset because there is less time for adaptation in the cardiovascular system and in the O₂ dissociation curve of hemoglobin (1).

1.1.1.3.2 Severity:

Mild anemia often produces no symptoms or signs but these are usually present when the hemoglobin is less than 9-10 g/dl. Even severe anemia (hemoglobin concentration as low as 6.0 g/dl) may produce remarkably few symptoms however, when there is very gradual onset in a young subject who is otherwise healthy. The elderly tolerate anemia less well than the young because of the effect of lack of

oxygen on organs when normal cardiovascular compensation (increased cardiac output caused by increased stroke volume and tachycardia) is impaired. Anemia in general is associated with a rise in 2,3 DPG in the red cells and a shift in the O₂ dissociation curve to the right so that oxygen is given up more readily to tissues. This adaptation is particularly marked in some types of anemia which either affect red cell metabolism directly (e.g. the anemia of pyruvate kinase deficiency which causes a rise in 2, 3-DPG concentration in the red cells) or which are associated with a low affinity hemoglobin (e.g. Hb S)(1).

1.1.1.3.3 Symptoms of anemia:

These are usually shortness of breath particularly on exercise, weakness and palpitation lethargy. In older subject symptoms of cardiac failure, angina pectoris, intermittent claudication or confusion may be present. Visual disturbances because of retinal hemorrhage may complicate very severe anemia, particularly of rapid onset (1).

1.1.1.3.4 Signs of anemia:

These may be divided into general and specific. General signs include pallor of mucous membranes which occurs if the hemoglobin level is less than 9-10g/dL. Conversely, skin color is not a reliable sign. A hyperdynamic circulation may be present with tachycardia, bounding pulses, cardiomegaly and systolic flow murmur especially at the apex. Particularly in the elderly, features of congestive heart failure may be present. Retinal hemorrhages are unusual. Specific signs are associated with particular types of anemia (e.g. koilonychia (spoon nails) with iron deficiency, jaundice with haemolytic or megaloblastic anemia, leg ulcers with sickle cell and other haemolytic anemias, bone deformities with thalassaemia major and other severe congenital haemolytic anaemias (1).

1.1.1.4 Lab diagnosis of anemia:

Diagnosis may be suspected on the basis of the history and examination but laboratory investigations are required for confirmation. Iron deficiency causes hypochromic microcytic anemia. The automated red cell analyzer generates a report with haemoglobin, MCV and MCH values below the normal range. There is a variation in red cell size (anisocytosis) reflected by a high red cell distribution width (RDW) (3).

1.1.2. The Pregnancy

Pregnancy is the state of carrying a growing embryo or fetus in the uterus. In mammals, pregnancy is defined as the period between implantation of fertilized egg (now called a zygote) in the wall of uterus and delivery or other termination. Some people consider conception, the moment when the sperm and the egg first meet in the fallopian tube, as the start of pregnancy, while the legal and medical definitions hold that pregnancy begins when the zygote implant in the uterine wall. Human Physiological anemia is the term often used to describe the fall in haemoglobin (HB) concentration that occurs during normal pregnancy. Blood plasma volume increase by around 1250ml, or 45%, above normal by the end of gestation and although the red cell mass itself increase by some 25% this still leads to a fall in Hb concentration. Values below 10g/dl are probably abnormal and require investigation (1).

1.1.2.1. Hematological changes during pregnancy:

During pregnancy, the total blood volume increase by about 1.5 liters, mainly to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery. Of this, around one liter of blood is contained within the uterus and maternal blood spaces of the placenta. Increase in blood volume is, therefore, more marked in multiple pregnancies and iron deficient states. Expansion of plasma

volume occurs by 10-15% at 6-12 weeks of gestation. During pregnancy, plasma renin activity tends to increase and atrial natriuretic peptide levels tends to reduce, though slightly. This suggests that, in pregnant state, the elevation in plasma volume is in response to an underfilled vascular system resulting from systematic vasodilation and increase in vascular capacitance, rather than primary blood volume expansion, which would produce the opposite hormonal profile instead (7).

A key to the diagnosis of iron deficiency anemia in pregnancy is the examination of the red cell indices. The earliest effect of iron deficiency on the erythrocyte is a reduction in cell size, MCV and in pregnancy, with the dramatic change in red cell mass and plasma volume, this is the most sensitive indicator of underling iron deficiency hypochromia and fall in the MCHC only appear with more severe degrees of iron depletion. Some woman start pregnancy with already established anemia due to iron deficiency or with grossly depleted iron store and they will quickly develop florid anemia with reduced MCV, MCH and MCHC (7).

1.1.2.2 Normal Complete blood count:

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: hemoglobin level, red blood cell (RBC) count, white blood cell (WBC) count and differential, platelet count 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 (3).

1.1.2.2.3 Hemoglobin:

Is the iron – containing oxygen-transport in the red blood cells of all vertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs) to the body tissues where it releases the oxygen to burn nutrients to provide energy to power the functions of the human, and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the human. (3)

1.1.2.2.4 Hematocrit and red cell indices:

The Hematocrit is a test that measures the volume of blood in percent that is comprised of the red blood cells. The three main RBC indices are used to determine the average size and hemoglobin content of the RBCs and they help determine the cause of anemia are mean corpuscular volume (MCV) the average size of the red blood cells expressed in femtoliters. MCV is calculated by dividing the hematocrit (as percent) by the RBC count in millions per microliter of blood, then multiplying by 10, mean corpuscular hemoglobin (MCH) the average amount of hemoglobin inside an RBC expressed in pictograms. The MCH is calculated by dividing the hemoglobin concentration in grams per deciliter by the RBC count in millions per microliter, then multiplying by 10 and mean corpuscular hemoglobin concentration (MCHC) the average of Hb in the RBCs expressed in percent. It is calculated by dividing the hemoglobin in grams per deciliter by the hematocrit, then multiplying by 100 (3).

1.1.2.2.5 White blood cell count:

The majority of CBC include both WBC count and an automated differential determines the percentage of each of the five types mature white blood cells. WBCs consist of two main subgroup the mononuclear cells and the granulocytic cells. Mononuclear cells include lymphocytes and monocytes. Granulocytes include neutrophils, eosinophil and basophils. Neutrophils are normally the most abundant WBCs .They measure 12-16 μm in diameter. The nucleus stains dark purple – blue,

and is divided into several lobes (usually three or four) consisting of dense chromatin, Eosinophils 14 – 16 µm in diameter and contain a blue nucleus that is segmented into two distinct lobes. The cytoplasm is filled with large refractile orange – red granules, Basophils, like eosinophils, are 14 -16 µm in diameter and have a blue nucleus that is bilobed. The cytoplasm of the basophil is filled with large dark blue – black granules that may obscure the nucleus, Lymphocytes are the second most abundant WBCs. They may be small (7-9µm in diameter) or large (12-16 µm in diameter). The nucleus is dark blue and is nearly round or slightly indented and the chromatin is clumped and very dense. The cytoplasm is medium blue and usually granular and Monocytes are the largest WBCs measuring 14-20 µm in diameter. They have a large irregularly shaped and folded blue nucleus with chromatin that is less dense than other WBCs. The cytoplasm is gray – blue with fine granules. (3)

1.1.2.2.6 Platelet count:

Platelets are disk – shaped structures formed by the detachment of cytoplasm from megakaryocytes. They aid in the coagulation process by attaching or adhering to the walls of injured blood vessels, where they stick together to form the initial platelet plug. The platelet count is most often measured by impedance counting but is performed manually when the platelet count is very low, platelet clumping is observed, or abnormally large (giant) platelets are present(3).

1.2 Literature Review

1.2.1 Iron deficiency anemia among pregnant women in developing countries

Study done in Iran by Barooti E, Sadeghirad B et al November 2010 all published papers in main national and international database were systematically searched for some specific keywords to find the related studies between the year 1993 and 2007. All published studies which had reported the prevalence of anemia were included in the study except studies on refugee, patients undergoing hemodialysis, patients with thalassemia or cancer or other selective subpopulations. Two trained reviews independently assessed the inclusive/exclusive criteria and the quality of the selected papers, summarized them and eventually analyzed the data. Ten eligible papers including 11,037 participants were entered into the analysis. The maximum and minimum reported prevalence rates of anemia during pregnancy were 4.3% and 21.5%, respectively. The overall estimate of anemia prevalence in Iranian pregnant women was 13.6 (95% CI: 8.3-18.9). Excluding the only out-layer from the meta-analysis, the overall estimated prevalence was 12.4% (95% CI: 9.6% - 17.9%). The prevalence of anemia in Iranian women during pregnancy is considerably lower than that of most EMBRO countries or the one reported by WHO for Iran (>40%) which had been performed on a small group 16 years ago. The lower prevalence rate of anemia in pregnant women versus the regional rates could be due to the improvements of the national health system and prenatal programs in recent years.

Study Done in Jordan by Mohammad A. Salahat and *et al.*, November 29, 2011 the study was conducted on 1030 pregnant women in the age of 16-40 years for the assessment of their hemoglobin status. One hundred pregnant women in their first trimester were selected from the whole sample to study the effect of their pregnancy on alkaline phosphatase activity. The overall prevalence of anemia and the mean hemoglobin (HB) concentrations in the investigated sample were found to be 56.7% and 9.8 ± 1.4 g/dL. The overall prevalence of anemia by duration of pregnancy was found to be 47.0% during the first trimester (mean HB concentration 11.0 ± 1.6

g/dL), 56.1 % during the second trimester (mean HB concentration 10.1 ± 1.3 g/dL) and 66.9% during the last trimester (mean HB concentration 8.7 ± 1.4 g/dL), which also, showed the highest prevalence of severe anemia. The overall prevalence of anemia by the number of pregnancies was found to be higher in Multipara women (64.0%) than among primigravida women (49.3%). The difference was statistically significant ($P < 0.01$) (9)

1.2.2 Iron deficiency anemia among pregnant women in un-developing countries

Study Done in Indonesia, by Ketut.T.S *et al.*, September 2002 the study was conducted among 1,684 pregnant women in 42 villages in Bali that were selected by probabilistic/proportional-to-size sampling technique. Two ml of venous blood were collected for hemoglobin estimation using an automatic hematology analyzer (Technician H-I), and serum ferritin examination using immunofluorescent technique. The WHO criterion for anemia in pregnancy was applied and serum ferritin < 20 $\mu\text{g/l}$ as cut-off point for iron deficiency. Data regarding risk factors were gathered using pre-designed questionnaires. The prevalence of iron-deficiency anemia in pregnant women was 46.2%. The range of hemoglobin levels was 4.1 -15.9 g/dl, with a mean of 11.05 g/dl (standard deviation 1.01 g/dl). Applying the WHO criterion, 778 pregnant women were classified as anemic, giving a prevalence rate of 46.2%. The relationship between prevalence of iron-deficiency anemia and patient age Prevalence is lower in the 20-35 year age group compared with the < 20 year age group and the > 35 year age group; these differences are not statistically significant ($p > 0.05$). The prevalence of anemia decreased with an increase in the level of education. Pregnant women with no formal education were associated with a significantly higher prevalence of iron-deficiency anemia. The prevalence of iron-

deficiency anemia was higher in the group without iron pills intake compared with the group with a regular intake of iron pills ($p < 0.05$) (10)

Study Done in Pakistan (Peshawar) by Hamzullah Khan *et al.*, in 2013 the study was Lady reading Hospital (PGMI-LRH) Peshawar. Period: 1 August 2012- 10 Dec 2012. A Total of 152 women attending the center were included. Of total 81(53%) of the females were having hemoglobin less than 11g/dl and 22% of the women had HCT<32% which as per criteria of the WHO were anemic at the time of presentation. While 29(19%) patients had low value of MCV (microcytic). In present study 81(53%) of the females were having hemoglobin less than 11g/dl and 22% of the women had HCT<32% which as per criteria of the WHO were anemic at the time of presentation. A local study reported that the prevalence of anemia (defined by the World Health Organization as hemoglobin <11.0 g/dL) in the study subjects was 90.5%; of these, 75.0% had mild anemia (hemoglobin from 9.0 to 10.9 g/dL) and 14.8% had moderate anemia (hemoglobin from 7.0 to 8.9 g/dL). Only 0.7% were 8 severely anemic (hemoglobin < 7.0 g/ dL) we also found that 29(19%) patients had low value of MCV (microcytic) (11)

Study Done in West Algeria (Sidi Beh Abbes Region) by Demmouche A, *et.al* in 2010 the study included 242 pregnant women attending MCH center in SidiBel Abbes region, West of Algeria for the assessment of their hemoglobin level. The overall prevalence of anemia (HB<11g/dL) was found to be 40.08 %. Classified in each trimester, the prevalence was 17.3%, 23.8% and 50.0% in the first, second and third trimester, respectively. According to severity of anemia 36.08% having mild 49.48% moderate and 14.43% severe anemia. The study shows that 46.39% of the subject had MCV values less than standard value of 75fl suggesting microcytic anemia. No correlations were found between the hemoglobin and the maternal obstetric characteristics, in particular not between hemoglobin concentration and

parity ($p=0.40$), between HB and number of abortion ($r=0.005$, $p=0.30$). Our study shows that age and parity were not a risk factor for anemia. Relation between maternal age and mean HB distribution. The age of the mother is no significantly associated with anemia. The majority of mothers (38.14%) who are less than 26 years old being anemic at the first antenatal visit. We found no correlation between the HB level and maternal age ($r = 0.17$, $p=0, 67$)(12)

Study Done in India (Karnataka), by Judith A. Noronha in 2008. The aim of the present study was to identify the prevalence of anemia among pregnant women attending ante-natal care units of selected hospitals of Udupi district during 2005-2006. About 1077 pregnant women were screened for anemia using cyanmethemoglobin method during the first ante-natal visit. The prevalence of anemia was found to be 50.14 per cent HB >11 g/dl which is nearly equivalent to the prevalence rate reported in the literature for Karnataka (13)

Study Done in Nigeria, by Erhabor.O, in (2013). The study include 55 pregnant women (subjects) and 33 apparently healthy non-pregnant women (controls). The mean age and range of subjects was 33.25 ± 11.50 and 18-40 years respectively The mean values of the hematology and anemia –related parameters among the pregnant subjects were; Hemoglobin (10.14 ± 1.45 g/dL), PCV ($30.567 \pm 4.492\%$), SI ($153.55 \pm 66.061 \mu\text{g/dl}$), TIBC ($4.33.18 \pm 97.248 \mu\text{g/dl}$), Serum Ferritin (32.9 ± 14.2 ng/mL) and TS ($7.69 \pm 28.84\%$). The overall prevalence of anemia based on WHO criteria (Hemoglobin <11 g/dL) and iron deficiency anemia based on Ferritin < 12.0 ng/mL and TS $<16\%$ was found to be 21.3%, 13.5% and 7.69% respectively among pregnant women studied. The mean MCV, MCH and MCHC observed among the pregnant subjects was significantly lower compared to those of non-pregnant controls (70.9 ± 11.5 fL, 25.4 ± 3.6 pg/cell and 31.9 ± 1.6 g/dl) compared to 84.9 fL ± 10.5 , 27.8 ± 3.4 pg/cell and 32.9 g/dl ± 2.0 g/dl) respectively. Blood film examination indicated

that (58.3%) of subjects that met the WHO definition of anemia (HB <11g/dl) showed a microcytic and hypochromic red cell picture. The prevalence of IDA was significantly higher in women in the 3rd trimester of pregnancy compared to the 2nd trimester (14).

Study Done in Bangladesh, by SM ZiauddinHyder in (2004), University of Toronto, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8, the study included 214 reportedly healthy pregnant women in their second trimester. Information on socio-economic status and reproductive history were obtained through home visits and venous blood samples were collected at antenatal care centers. Hemoglobin concentration (HB) was measured by HemoCue .The prevalence of anemia (HB<11g/dl) was 50 %(15).

1.2.3 Iron deficiency anemia among pregnant women in Sudan

Study Done in Sudan, by Esam G Abdelrahman in (2012), the study was a cross-sectional descriptive study was carried out at the antenatal clinic of Khartoum Hospital. Among 194 pregnant women with a gestational period of 21.4 ± 6.5 weeks, 57 (29.4%) had IDA according to serum ferritin levels (<15 $\mu\text{g/l}$) (16)

1.3 Rationale

Anemia is a cause a high morbidity and mortality for pregnant women and for their babies. Iron deficiency the most commonest cause of anemia during pregnancy. The prevalence of iron deficiency among anemic pregnant ladies in Gedaref has not been studied. Evaluation of hematological values among anemic pregnant women give valuable information about iron status that use as predictive for early occurrence of anemia during pregnancy.

1.4 Objectives

General objectives

To determine the some hematological parameters and iron profile among anemic pregnant women in Al-Gadarif State.

Specific objectives

- To determine full blood count among anemic pregnant women (Hb, HCT, TRBC`s, TWBC`s platelet count MCV, MCH and MCHC).
- To determine iron profile among anemic pregnant women (serum iron, serum ferritin and TIBC).
- To compare between test and controls.

Chapter Two

Material and Methods

2.1 Materials

2.1.1 Study design:

This cross sectional study was conducted from December 2012 to June 2014 in Sudanese anemic pregnant women in EI-Gadarif state maternity hospital.

2.1.2 Study population:

Pregnant women with a level less than 11 mg/dl.

2.1.3 Sample size:

200 samples of venous blood were collected from anemic pregnant women as cases by random selection method and 100 venous blood samples were collected from non-pregnant women.

2.1.4 Inclusion criteria:

Anemic pregnant women were included in this study.

2.1.5 Exclusion criteria

Pregnant women have another disease.

2.1.6 Ethical consideration:

Informed consent from each anemic pregnant woman and all participants aware about the research.

2.2 Methods

2.2.1 Laboratory requirements (equipment & reagents):

1. Automated Hematological analyzer Sysmex KX 2IN for determination of complete Blood Count.
2. Microscope.
3. Leshman stain for preparation of thin blood films.
4. Slides cover glass, oil.
5. Test tube 5ml.
6. Pasture pastauer.
7. EDTA tube container.
8. Centrifuge.
9. 70% alcohol (ethanol).
10. Cotton and tourniquet.
11. Syringes

2.2.2 Sample collection

Five ml of blood was collected under sterile conditions, 2.5ml of blood was drained into K2EDTA container to be used for complete blood count (CBC) by using automated hematological analyzer (sysmex21) and for preparation of thin blood film for RBCs morphology the remaining 2.5ml of blood drained in the sterile plain container to obtain clotted sample which was immediately centrifuged to harvest serum for determination of serum iron and ferritin using selectra-XL

2.2.3 Complete blood count (CBC):

CBC and blood film examination usually indicate wherever there is any abnormalities in blood cells, SysmexKX 2IN (automated hematological analyzer) was used.

2.2.4 Principle of automated analyzer system (sysmex):

The counting of cellular elements in blood sample is done with the impedanceometry technique. This technique is based on the modification of the impedance of a calibrated aperture soaked in an electrolyte and going through a constant course delivered by two electrodes located on both sides of the aperture. A tube with a small aperture on the wall is immersed into a beaker that contains particles suspended in a low concentration electrolyte. Two electrodes, one inside the aperture tube and one outside the aperture tube but inside the beaker, are placed and a current path is provided by the electrolyte when an electric field is applied. The impedance between the electrodes is then measured. The aperture creates what is called a “sensing zone” Particles in low concentration, suspended in the electrolyte, can be counted by passing them through the aperture. As a particle passes through the aperture, a volume of electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short-term change in the impedance across the aperture. This change can be measured as a voltage pulse or a current pulse. The pulse height is proportional to the volume of the sensed particle. If constant particle density is assumed, the pulse height is also proportional to the particle mass. This technology thus is also called aperture technology. Using count and pulse height analyzer circuits, the number of particles and volume of each particle passing through the sensing zone can be measured. If the volume of liquid passing through the aperture can be precisely controlled and measured, the concentration of the sample can be determined.

2.2.5 Preparation of thin blood film:

Clean slide wiped immediately before use. Small drop of fresh anticoagulant blood was placed in the center line of slide about 1 to 2cm at an angle of 45C° to slide and moved back to make contact with the drop, the drop was spread quickly along the line of contact of spreader with slide, the film was left to air dry.

2.2.6 Staining film:

The slide was flooded with leishmans stain on the staining rack, after 3 minutes double volume of buffer was added for 7 minutes. And then washed with tap water and left to air dry (17).

2.2.7 Examination of blood film:

Blood films were examined for cells size, shape, hemoglobin content and distribution, leukocytes deferential and abnormality, staining properties, and inclusions bodies. The films were examined by x10 magnification of eye lens for staining quality and x40 and x100 for the blood cells differentiation.

2.2.8 Selectra– XL background and principles:

Selectra – XL analyzer is an automated analyzer for in vitro diagnosis specially designed for performing Biochemical (and turbidometric clinical analysis). The instrument is controlled online in real time from an external dedicated programmed computer (PC) the principle of selectra XL depends on the absorbance of ultraviolet light or visible light by the samples. This measurement technique is used for determining concentrations of iron and ferritin in serum, the sample is mixed with reagent first before measuring the amount of light absorbance because interfering elements in the sample, this process was automatically done by instrument after calibration. The result is shown immediately after each measurement. The flexibility of the samples and reagents rack system

enable the perfect adjustment of the capacity of the analyzer to the specific needs of each laboratory.

2.2.9 Serum iron principle and value:

Transferring-bound ferric irons in the sample are released by guanidinium and reduced to ferrous by means of hydroxylamine. Ferrous reacts with ferrozine forming a colored complex that can be measured by spectrophotometry the determination of serum iron level is used for the differential diagnosis of the common disorders of iron metabolism(18).

2.2.10 Serum ferritin principle and value:

Serum ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies. The agglutination of the latex is proportional to the ferritin concentration and can be measured by turbidimetry. Determination of serum ferritin concentration used as an indicator for hemopoietic tissue iron and aid in diagnosis of iron deficiency and iron overload (19)

2.2.11 Total Iron binding Capacity (TIBC) & Transferrin Saturation:

The transferrin in the specimen is saturated with iron by exposure to excess ferric ions; the unbound iron is removed by addition of light magnesium carbonate and centrifugation. The iron bound to protein in the specimen is measured by the same principle applied to serum iron described above (18).

2.2.12 Statistical analysis:

Statistical package for social Science (SPSS version 11.5) software program was used, to obtain mean, standard deviations frequencies and

independent T test was use to compare between the parameters of case and control.

Chapter Three

The Results

Results

A total number of 200 anemic pregnant women (study group) were enrolled into the study from the period December 2012 to June 2014. The study group had an age range between 18 to 40 years.

As indicated in figure (3.1)

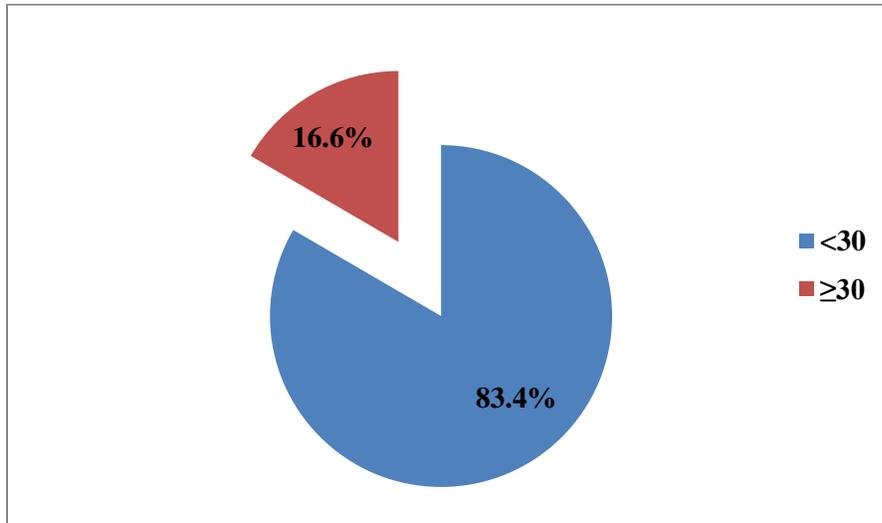


Fig: (3.1) Distribution of anemic patients by age

The distribution of anemic pregnant women to their age <30 years are 83.4% and ≥ 30 are 16.6% (table3.1)

Table 3.1 Show General Characterizes among anemic pregnant women

| Characteristics | | Patients N (%) |
|--------------------------|------------|-----------------------|
| Age group | <30 | 166(83.4) |
| | ≥30 | 34(16.6) |
| Education level | Primary | 111(55.8) |
| | Secondary | 77(38.7) |
| | University | 11(5.5) |
| Living level | Low | 158(79.4) |
| | Normal | 41(20.6) |
| Abortion | | 91(45.7) |
| Iron pills intake | | 8(4) |
| Pregnancy number | <4 times | 79(39.7) |
| | ≥4 times | 120(60.3) |

N=number of patients

As shown in figure (3.2) average hemoglobin and hematocrit among study group and control group. The study group revealed mean of Hb and HCT of (7.66 ± 1.31 and 24.4 ± 15.6) compared with control group of (12.46 ± 2.12 and 38.78 ± 5.84) (figure 3.2 Table 3.2).

Our study conducted that hemoglobin and hematocrit decreased in study group compared control group. The P.value<0.001 is highly significantly different (table 3.2)

Table 3.2 Comparison between mean value of CBC among pregnant women and controls

| Risk Factor | Patients Mean\pm SD | Controls Mean\pm SD | P-value |
|--|---|---|----------------|
| Hematocrit (%) | 24.4 \pm 15.16 | 38.78 \pm 5.84 | <0.001 |
| Haemoglobin (g/dL) | 7.66 \pm 1.31 | 12.46 \pm 2.12 | <0.001 |
| Platelets | 159.63 \pm 45.55 | 175.76 \pm 62.59 | <0.05 |
| White Blood Cell (WBCs) | 8.27 \pm 2.53 | 7.82 \pm 2.66 | >0.05 |
| Red Blood Cell (RBCs) | 3.29 \pm 0.63 | 5.01 \pm 0.71 | <0.001 |
| Mean Cell Haemoglobin Concentration (g/dl) | 25.15 \pm 2.83 | 32.97 \pm 3.92 | <0.001 |
| Mean Cell Haemoglobin (pg/cell) | 23.07 \pm 2.59 | 27.39 \pm 3.76 | <0.001 |
| Mean Cell Volume (fL) | 73.42 \pm 6.87 | 79.34 \pm 14.87 | <0.001 |

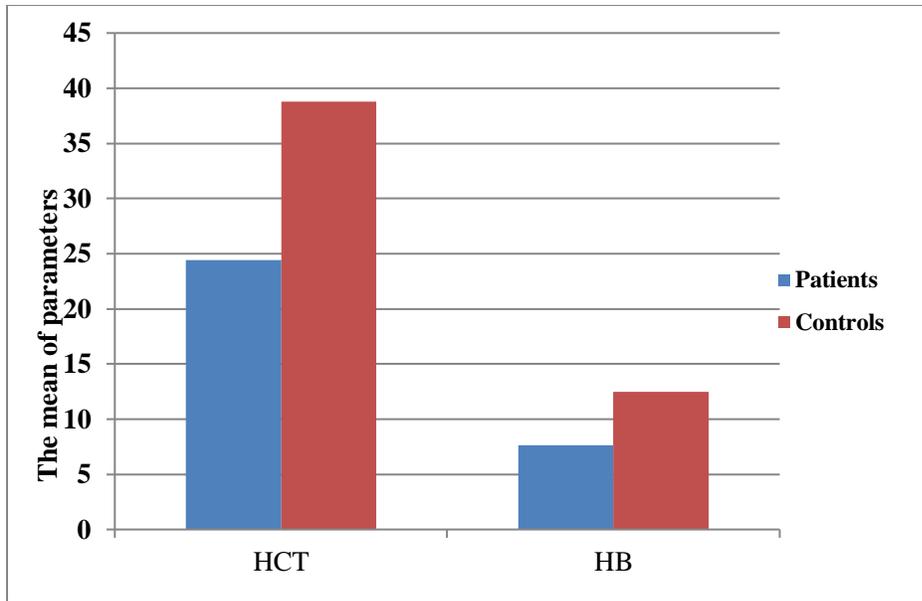


Fig: (3.2) Average of HB and HCT among pregnant women and controls

As shown fig (3.3) average of mean value of platelet WBCs and RBCs were assayed for each group, the study group revealed mean platelet.

Our study conducted platelet is decreased in pregnant women shows decrease significant different with control group. The P.value<, 0.05.

WBCs is no significant different with control group P.value>0.05)

The RBCs decreased in number in study group is highly different with control group the P.value<0.001.

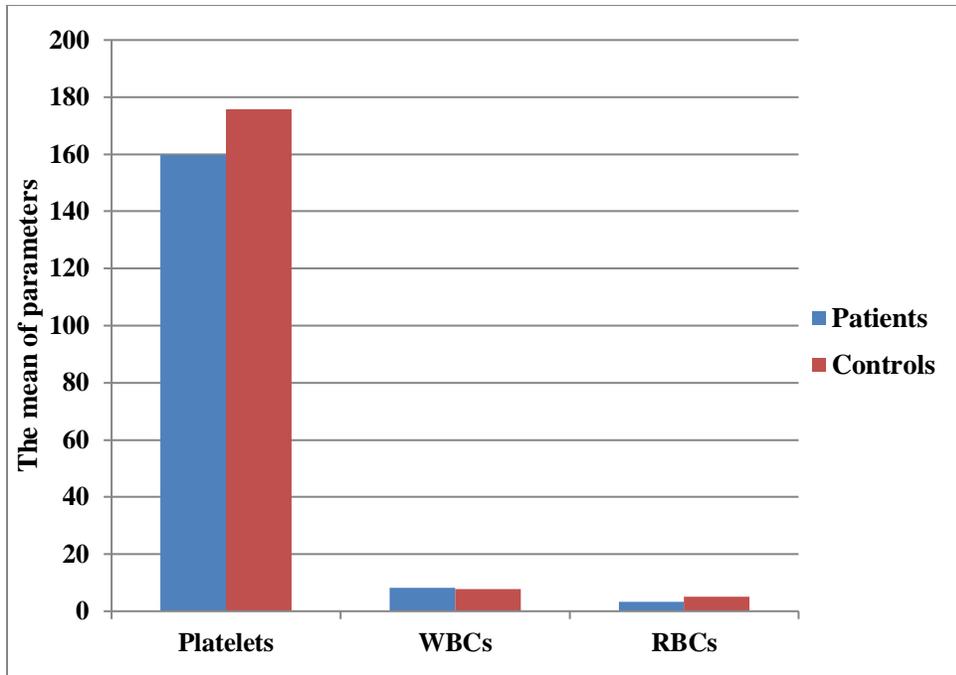


Fig (3.3) Average of mean value platelets, WBCs and RBCs pregnant women and controls.

As shown figure (3.4) average of MCV, MCH, MCHC among study group and control group were assayed of each group. The study group revealed mean of (MCV, MCH, MCHC) were $(73.42 \pm 6.87, 23.07 \pm 2.59, 25.15 \pm 2.83)$ compared with control group.

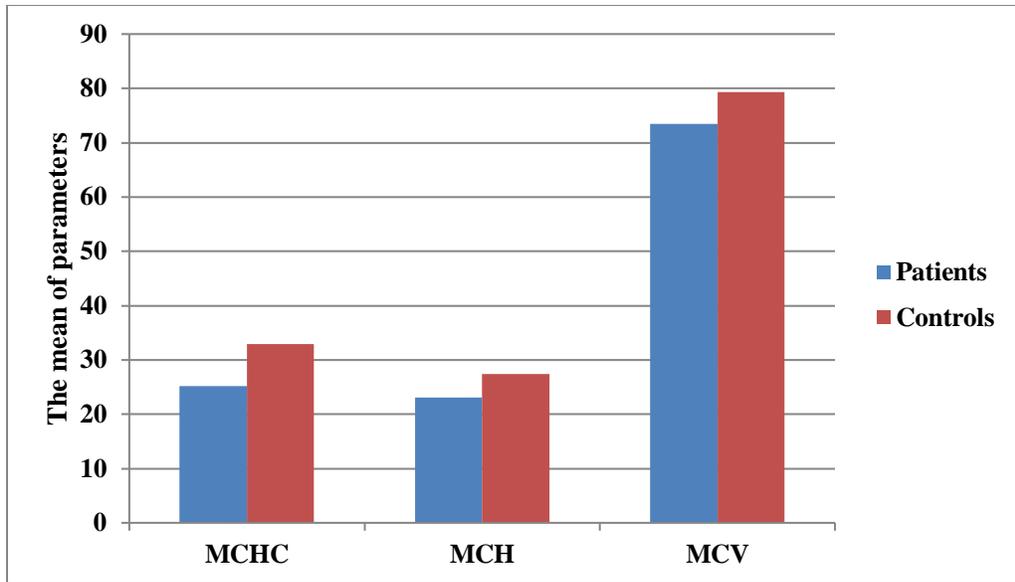


Fig (3.4) Average of MCHC, MCH and MCV among pregnant women and controls

figure (3.5) shows average of iron profile among anemic pregnant women and controls group (serum iron, serum ferritin, total iron binding capacity) were assayed for each group, the study group revealed mean of -serum iron, serum ferritin and total binding capacity were (114.52 ± 36.41 , 105.94 ± 34.86 and 285.29 ± 40.93)(fig 3.2 and table 3.2). Our study conducted that serum iron and serum ferritin were decrease in anemic pregnant women compared with healthy control group. The P.value <0.001 is highly significantly different however the average of total iron biding capacity increased in the study group compared with healthy control group. The value <0.001 is highly significantly different table (3.2)

Table 3.3 Comparison between the main value of Iron profile among pregnant women and controls

| Risk Factor | Patients Mean± SD | Controls Mean± SD | P-value |
|-------------------------------------|------------------------------|------------------------------|----------------|
| Serum Iron(μg/dl) | 17.14±3.51 | 114.52±36.41 | <0.001 |
| Serum Ferritin | 9.45±1.90 | 105.94±34.86 | <0.001 |
| Total Iron Binding Capacity (μg/dl) | 428.02±66.47 | 285.29±40.93 | <0.001 |

P-value<0.001 highly significantly different

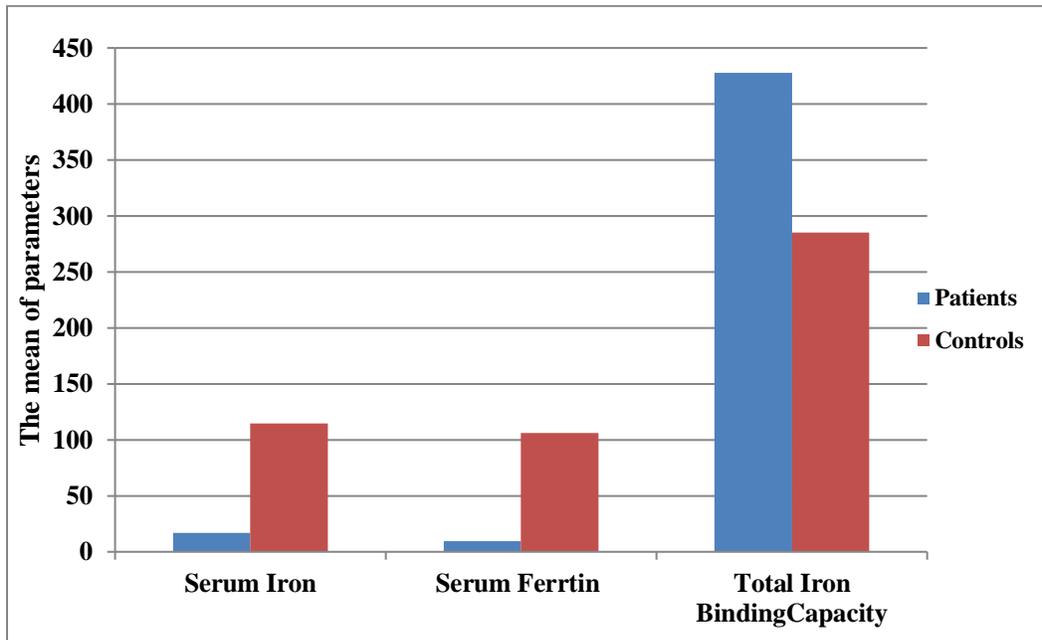


Fig: (3.5) Average of iron profile among pregnant women and controls

Table 3.4 Comparison between average iron profile and HB according to age group

| Age group | Serum Iron (µg/dl) Mean± SD | Serum Ferritin Mean± SD | Total Iron Binding Capacity (µg/dl) Mean± SD | Haemoglobin (g/dL) Mean± SD | Hematocrit (%) Mean± SD |
|------------------|--|------------------------------------|---|--|------------------------------------|
| <30 | 17.24±3.59 | 9.49±1.94 | 427.20±65.74 | 7.67±1.35 | 24.82±16.55 |
| ≥30 | 16.54±3.03 | 9.18±1.68 | 434.31±70.86 | 7.54±1.06 | 22.43±3.34 |
| P-value | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

In table (3.5) shows average of iron profile and hemoglobin according to level of education, the prevalence of iron profile and Hb compared with egducation level these difference are no statistically significant different (p>0.05).

In table 3.5 no correlation between educations level hemoglobin, hematocrit, serum iron, serum ferritin and total iron binding capacity (P. Value >0.005) (Table 3.5).

Table 3.5 Comparison between average of iron profile and HB according to level of education

| Level of education | Serum Iron (µg/dl) Mean± SD | Serum Ferritin Mean± SD | Total Iron Binding Capacity (µg/dl) Mean± SD | Haemoglobin (g/dL) Mean± SD | Hematocrit (%) Mean± SD |
|---------------------------|--|------------------------------------|---|--|------------------------------------|
| Primary | 17.59±3.61 | 9.67±1.92 | 430.64±63.38 | 7.77±1.32 | 25.50±19.95 |
| Secondary | 16.68±3.43 | 9.19±1.89 | 425.14±70.39 | 7.49±1.25 | 23.09±4.31 |
| University | 15.80±2.36 | 8.83±1.45 | 428.0±73.97 | 7.59±1.53 | 22.73±4.59 |
| P-value | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

Table (3.6) shows comparison between hemoglobin and hematocrite according living level, the prevalence of serum iron, serum ferritin and hemoglobin) this differences are decreased statistically significant (P<0.05)

Table 3.6 Comparison between average of iron profile according living level

| Living level | Serum Iron (µg/dl) Mean± SD | Serum Ferritin Mean± SD | Total Iron Binding Capacity (µg/dl) Mean± SD | Haemoglobin (g/dL) Mean± SD | Hematocrit (%) Mean± SD |
|---------------------|--|------------------------------------|---|--|------------------------------------|
| Normal | 17.42±3.58 | 9.60±1.91 | 425.81±62.47 | 7.76±1.30 | 25.02±16.86 |
| Low | 15.96±2.99 | 8.80±1.75 | 438.3±80.33 | 7.24±1.25 | 22.13±4.41 |
| P-value | <0.05 | <0.05 | >0.05 | <0.05 | >0.05 |

Table (3.7) shows average of iron profile and hemoglobin and hematocrit according the abortion, 45.7% of study group had abortion, and the difference are no statistically significant.

Table 3.7 Comparison between Average of Iron profile and Haemoglobin according to abortion women

| Abortion | Serum Iron (µg/dl) Mean± SD | Serum Ferritin Mean± SD | Total Iron Binding Capacity (µg/dl) Mean± SD | Haemoglobin (g/dL) Mean± SD | Hematocrit (%) Mean± SD |
|-----------------|--|------------------------------------|---|--|------------------------------------|
| Yes | 17.12±3.73 | 9.42±2.04 | 425.67±64.50 | 7.69±1.31 | 23.23±4.16 |
| No | 17.13±3.32 | 9.46±1.78 | 430.69±68.34 | 7.62±1.31 | 25.43±20.26 |
| P-value | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

Table (3.8) average of iron profile and hemoglobin according to number of pregnancy in the study group the pregnancy number ≥ 4 times (33%) and < 4 time (83.4). the difference are no statistically significant P.value>(0.05).

No correlation between hemoglobin and number of abortion (P.value>0.05) and hemoglobin and number of pregnancy (P.value>0.05) hemoglobin and number of pregnancy (P.value>0.05). Hemoglobin and education and education level P.value> (0.05) (Table 3.8).

Table 3.8 Comparison Average of Iron profile and Haemoglobin according to the number of pregnancy.

| Number of pregnancy | Serum Iron (µg/dl) Mean± SD | Serum Ferritin Mean± SD | Total Iron Binding Capacity (µg/dl) Mean± SD | Haemoglobin(g/dL) Mean± SD | Hematocrit (%) Mean± SD |
|----------------------------|------------------------------------|--------------------------------|---|-----------------------------------|--------------------------------|
| <4 times | 17.33±3.51 | 9.56±1.99 | 428.88±72.98 | 7.60±1.31 | 25.89±23.51 |
| ≥4 times | 16.99±3.51 | 9.36±1.84 | 428.03±62.21 | 7.68±1.31 | 23.46±4.39 |
| P-value | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

Blood film examination indicated show the red blood cell microcytic hypochromic.

All blood film examination for study group shows microcytic hypochromic.

Table 3.9 Frequency of Morphological pattern among anemic patients and controls

| Morphological pattern | Samples N (%) |
|--|----------------------|
| Microcytic hypochromic among anaemic patients | 200(100) |
| Normocytic normochromic among controls | 97(100) |

Statistical analysis

Statistical analyses were conducted using SPSS (version 11.5; SPSS) software. Data were expressed as mean \pm standard deviation. Comparisons between anaemic patients and controls were made using the Student's t-test for parametric data and one way ANOVA. Descriptive analyses of percentages of categorical variables were reported. An alpha value of < 0.05 denoted a statistically significant difference in all statistical comparison.

Chapter Four

Discussion, Conclusion and
Recommendation

4.1 Discussion:

Anemia associated with pregnancy is a public health problem and is estimated to affect 1.3 to 2.2 billion person worldwide, justification for the screening of pregnant women for iron deficiency anemia with increased risk for preterm delivery and other complications. This present study we have investigated the prevalence of anemia among pregnant women attending Al-Gedrif Maternity Hospital.

The study explained the hemoglobin in anemic pregnant women was significantly lower than value of control. The mean hemoglobin value is 7.66 g/dl. The mean hemoglobin in the study group <11 g/dl. This similar with study in Indonesia (8) and similar with study in Jordan (9) and similar with study in India (12).

In the study observed low value of serum ferritin. This similar with the study in Sudan (13) and similar with study in Nigeria (14). In the study observe high value of TIBC. Thee similar with study in Nigeria(14).

The mean value of MCV, MCH and MCHC in this study were decreased. This similar with study on Pakistan (Peshwar) (10), and similar with the study in Algeria (11) and similar with study in Nigeria (14).

The study observed the distribution of anemic pregnant women to their age less than 30 years are 83.4% and more than 30 years are 16.6% and observed no correlations between hemoglobin and age. That similar with the study in Indonesia (8).

The distribution of anemic pregnant women according to education level primary (55.8%), secondary (38.7%) and University (5.5%). We observed no statistical different between mean value of hemoglobin and education level. It is not similar with the study in Indonesia (8).

Frequency in anemic pregnant women according to pregnancy number less than four times 39.7% and more than four times was (60.3%) we observed no statistically

different between hemoglobin and number of pregnancy it is not similar with the study in Jordan (9).

We observed no statistically different between mean value of hemoglobin and age group (P.value > 0.05). These similar with study in Algeria²⁷ and similar with study in Indonesia²⁴.

The examination of blood film picture is microcytic hypochromic is similar with the study in Nigeria³⁰ and similar with study in Pakistan (10)

4.2 Conclusion

*Hemoglobin, Hematocrit, MCV, MCH, MCHC and RBCs were decreased in pregnant women show highly statistically different among control group, the platelet decreased shows different significant.

*Serum iron and serum ferritin were decreased in the pregnant women *Show statistical significant different between study group and control group.

4.3 Recommendations:

On the basis of this study and review of other studies recommended

*Hb count must be regularly measured in pregnant women.

*The importance of a balance diet and iron-rich food.

*Improve the national health system.

*Improve the living conditions.

References and Appendix

Appendices

Appendix (1) Questionnaire

The National Ribat University College of Graduate Studies and Scientific Research

Questionnaire

Serial Number:

Age

Name:

Level of Education:

Living level:

No. of pregnancy:

No. of abortion:

Hematological investigation result:

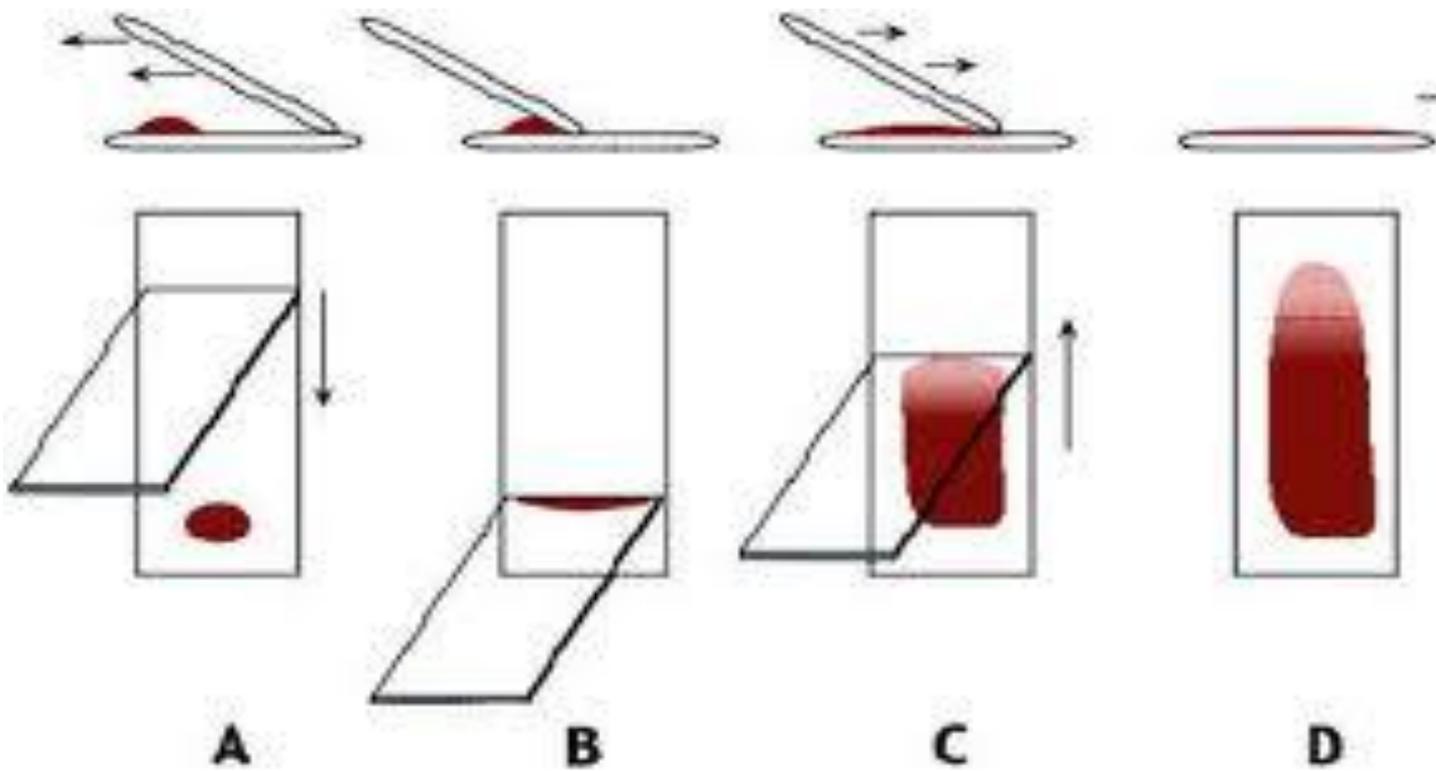
| Hb | HCT | MCV | MCH | MCHC | RBCs | TWBCs | Plts |
|------|-----|-----|-----|------|------|-------|------|
| g/dl | % | fl | pg | g/dl | /cmm | /cmm | cmm |
| | | | | | | | |

Biochemical Investigation results

| Serum iron | Serum ferritin |
|------------------|-----------------|
| $\mu\text{g/dl}$ | $\mu\text{g/l}$ |
| | |

Appendix (2) Color Plates

Preparation of a thin blood film



References

1. Hoff brand AV. Erthropoiesis and general aspects of anemia In: Hoff Brand AV, Pttit JE, Moss PAH, editors Essential Hematology, 4nd Edn, Blackwell Science: 2001. P.19-24.
2. Alhossian A. Khalafallah, Amanda E. Dennis. Iron deficiency anemia In: pregnancy and post partum pathophysiology and effect of oral versus intravenous iron therapy. Journal of pregnancy (2012); volume 2012 (2012): 1-10.
3. Martin RH. The anemia In: Martin RH, Howard, Peter JH, editors. Hematology an illustrated color text. 3^{ed} Edn.Chuchill Livingstone elsevier since 2008. P. 5-25.
4. Knapp DD. Erthrocyte abnormalities. In: William ED. editor. Clinical hematology. Oxford since 2003. P.125-135.
5. Hillman RS. Iron deficiency anemia. In: Hillman RS, Kenneth A.Ault, Henery m. Rinder editors. Hematology in clinical practice a guide to diagnosis and management 4th Edn. New York: Mc Graw – Hill Science; 2005. P. 53-65
6. Frank F. Hypochromic anemia. In: Frank F, Colin CM, David Penington and Bryan R. editors. De Grouchy Clinical Hematology in medical practice. 5^{ed} Edn. Melbourne since; 2005. P.37-43.

7. Surabhi Chandra, Anil Kumar, Sanjay, and Arvind Kumar Vaish. Physiological change in hematological parameters during pregnancy. Indian journal of hematology and blood transfusion; 2012 28(3) 144-146.
8. Brootie E, Mohammad MD and Behnam PD. prevalence iron deficiency anemia among irainion pregnant women. J Repord infertile 2010; 11(1):17-24
9. **Ketut S, Tjok GD, Made S, and Imade B.** A profile of risk factors and Epidemiology. British journal of Medicine and Health 2002; 1(4): 47-51.
10. **Mohammad A and Abdallah II** Prevalence of anemia among Jordanian pregnant women and the effect of early pregnancy on alkaline phosphate activity. Jordan Journal of Biological 2012; 5(1) 65-70.
11. **Hamzallah K, Khalid K, Fazie R and Aamir N.** Iron deficiency anemia Red cell distribution with and Red cell in third trimester of pregnancy. The professional Medical journal 2014; 2(1)100-105.
12. **Demmoche A, KhelilsA and Moulessehoul S** Anemia among pregnant women in Sidi Bel Abbes region (West Algeria) an Epidemiological study Blood Disord Transfuse J 2011; 2(3) 1-6

- 13. Judith AN, Aparna B and H Vinod B** Prevalence of anemia among pregnant women. A community based study in UDUPI District Health and population J. 2008; 31(1): 31-45.
- 14. Ehbor O, Isaac A, Isha A and Udomah FP.** Iron deficiency anemia among antenatal women in Sokoto, Nigeria. British Journal of medical and health 2013; 1(4):47-57
- 15. Ziauddin H, Lors A.P and Mustae C,** Anemia and iron deficiency during pregnancy in Bangladesh. J. Public Health Nutrition 2004; 7(8):1065-1070.
- 16. Abdelrahman EG, Imad RM, Leana M Elbashir and Ishag Adam** Red blood Cell Distribution width and iron deficiency anemia among pregnant Sudanese women. Diagnostic pathology J 2012; 7:168
- 17. Lewis SM. preparation of blood film. In: Lewis SM Bain B & Bates I editors. Dacie and Lewis Practical Hematology 10nd Edn. England: Elsevier Ltd since 2006. P.35-77.**
- 18. Dacie JV Lewis SM.** Iron Deficiency anemia. In: Dacie JV and Lewis SM. editors. Practical hematology 8^{ed} Edn. Churchill Livingstone: Douglas Mcnaughton since; 1995.p. 473 – 447.