

Chapter I

1. Introduction and Literature review

1.1 Introduction

Anemia is a condition that occurs when the red blood cells do not carry enough oxygen to the tissues of the body. Anemia affects all population groups. However, the most susceptible groups are pregnant women and young children. In the milder form, anemia is “silent”, without symptoms. In the more severe form, anemia is associated with fatigue, weakness, dizziness and drowsiness. The signs include loss of normal color in the skin (in fair skinned people) and also in the lips, tongue, nails, beds and the blood vessels in the white of the eyes. Without treatment, anemia can worsen and becomes an underlying cause of chronic ill health, such as impaired fetal development during pregnancy, delayed cognitive development and increased risk of infection in young children, and reduced physical capacity in all people. Low birth weight infants, young children and women of childbearing age are at risk of anemia. Women of childbearing age need to absorb 2-3 times the amount of iron required by men or older women (World Health Organization.2000).

Iron deficiency anemia (IDA) is the most common nutritional disorder in the world, as it is in the Eastern Mediterranean Region (EMR). A total of 149 million people in the EMR are iron deficient or anemic according to the World Health Organization (WHO) criteria (INACG, WHO, UNICEF 1998). Eighty three million are women (World Health Organization Report1992) . In fact the prevalence of anemia in the Gulf Region ranged from 15 to 48% in women of childbearing age (Verster A, *et al* .1995).

In Saudi Arabia the percentage was 30 to 56%. A cross-sectional study conducted in Riyadh City among schoolgirls showed that IDA prevalence was 40.5% among female adolescents (16-18) years old (Al-Shehri S.1996) . As pregnancy related anemia is linked with a high risk of maternal and fetal deaths as well as increasing prenatal mortality, the control of anemia in women of childbearing age is therefore a public health priority (WHO, UNICEF 1995).

Fortunately, anemia has received a great deal of attention in international health forums WHO/UNICEF jointly adopted new goals for the 1990, aiming amongst other things to control iron deficiency by the turn of the century . The international conference on nutrition (ICN) held in Rome, in December 1992, adopted the nutritional goals of the world summit for children, one of which was to reduce by one third (of the 1990 levels) the prevalence of IDA among women of child bearing age (INACG, WHO, UNICEF 1998). The aim of this study is to determine the risk factors for IDA among Saudi women of childbearing age.

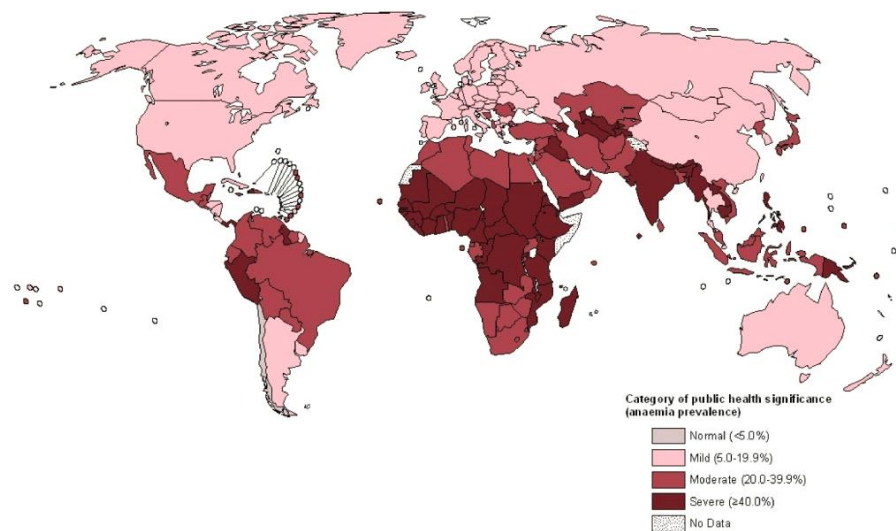


Figure (1-1) Anemia as a public health problem by country: Non pregnant women of Reproductive age. Adapted from (de Benoist ,et al .eds. prevalence of anemia).

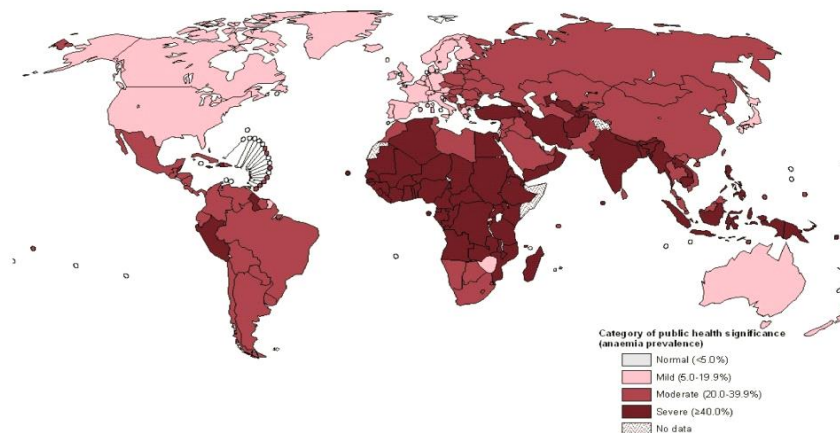


Figure (1-2) Anemia as a public health problem by country: Pregnant and

Adapted from (de Benoist ,*et al* .eds. prevalence of anemia) .

1.2 General introductions to anemia's

Anemia is defined as a reduction in the hemoglobin concentration of the blood. Although normal values can vary between laboratories, typical values would be less than 13.5 g/dl in adult males and less than 11.5 g/dl in adult females. From the age of 2 years to puberty, less than 11.0 g/dl indicates anemia. As newborn infants have a high hemoglobin level, 14.0 g/dL is taken as the lower limit at birth (.Hoffbrand, *et al*. 2006). It is important to recognize that the normal ranges for hemoglobin, hematocrit, and red blood cell indices are different for infants, children, and adults. In addition, with the aging process, modest changes in red blood cell mass occur in adults. Knowledge of age and gender appropriate normal values is important in the evaluation of anemia. For instance, in healthy men, there is a modest decline in hemoglobin with age of about 1 g/dl between the ages of 70 and 88 years, in part due to the decreased production of androgens. Only a minimal decrease in hemoglobin occurs between these ages in healthy women (about 0.2 g/dl) Table (1-1). Physician awareness of the expected change in hemoglobin in older men and its relative stability in older women can help

avoid unnecessary laboratory investigations in older men and correctly initiate further diagnostic evaluations in older women with mild anemia (Ronald Hoffman, *et al.* 2008).

Table (1-1): Normal red blood cell values.(Ronald Hoffman, *et al.* 2008)

Age	Hemoglobin mg/dL		Hematocrit (%)		Red Cell Count ($10^{12}/L$)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
	Mean	-2SD	Mean	-2SD	Mean	-2SD	Mean	-2SD	Mean	-2SD	Mean	-2SD
Birth (cord blood)	16.5	13.5	51	42	4.7	3.9	108	98	34	31	33	30
1–3 days (capillary)	18.5	14.5	56	45	5.2	4.0	108	95	34	31	33	29
1 week	17.5	13.5	54	42	3.1	3.9	107	88	34	28	33	28
2 weeks	16.5	12.5	51	39	4.9	3.6	105	86	34	28	33	28
1 month	14.0	10.0	43	31	4.2	3.0	104	85	34	28	33	29
2 months	11.5	9.0	35	28	3.8	2.7	96	77	30	26	33	29
3–6 months	11.5	9.5	35	29	3.8	3.1	91	74	30	25	33	30
0.5–2 years	12.0	11.0	36	33	4.5	3.7	78	70	27	23	33	30
2–6 years	12.5	11.5	37	34	4.6	3.9	81	75	27	24	34	31
6–12 years	13.5	11.5	40	35	4.6	4.0	86	77	29	25	34	31
12–18 years												
Female	14.0	12.0	41	36	4.6	4.1	90	78	30	25	34	31
Male	14.5	13.0	43	37	4.9	4.5	88	78	30	25	34	31
18–49 years												
Female	14.0	12.0	41	36	4.6	4.0	90	80	30	26	34	31
Male	15.5	13.5	47	41	5.2	4.5	90	80	30	26	34	31

1.2.1 Classification of anemia

Anemia can be approached from two perspectives: morphologic and pathophysiologic (also called functional or kinetic) (William. 2002).

1.2.1.1 Morphological classification

The most useful classification is that based on red cell indices (Table 1-2) and divides the anemia into microcytic, normocytic and macrocytic as well as suggesting the nature of the primary defect, this approach may also indicate an underlying abnormality before overt anemia has developed. In the newborn for a few weeks the MCV is high but in infancy it is low (e.g. 70 fL at 1 year of age) and rises slowly throughout childhood to the normal adult

range. In normal pregnancy there is a slight rise in MCV, even in the absence of other causes of macrocytosis. (Table 1-3).

Table (1-2): The classification of anemia based on red blood cell indices.(Hoffbrand , *et al.* 2006)

Microcytic. hypochromic	Normocytic. normochromic	Macrocytic
MCV <80fL	MCV80-95fL	MCV>95fL
MCH <27 pg	MCH>27pg	

MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

Table (1-3): The classification of anemia based on morphology (erythrocyte size). (F. Kern William. 2002).

Microcytic	Normocytic	Macrocytic
Iron deficiency	Anemia of chronic disease (most cases)	Megaloblastic anemia: folate or cobalamin deficiency
Thalassemia	Iron deficiency (early)	Hemolytic anemia (Reticulocytosis)
Sideroblastic anemia	Anemia of renal disease	Liver disease
Anemia of chronic disease (severe cases)	Combined nutritional deficiency: iron plus folate or cobalamin	Hypothyroidism
	Marrow failure , Hypothyroidism	Myelodysplasia

1.2.1.2 Etiological classification

The first step is to categorize the erythropoietic abnormality as one of three functional defects:

A failure in red blood cell production, an abnormality in cell maturation, or an increase in red blood cell destruction. This first step relies on the CBC and reticulocyte index. As shown in figure 1-2, defects in production (hypoproliferative anemia) are characterized by a low reticulocyte production index coupled with little or no change in red blood cell morphology. Maturation disorders demonstrate a low reticulocyte production index together with either

macrocytic or microcytic red blood cell morphology. In contrast, patients with increased red blood cell destruction owing to hemolysis show a compensatory increase in the reticulocyte index to levels greater than three times normal and red blood cell morphology that may or may not be distinctive for the disease process (Hillman,*et al* 2005).

This first step in classifying anemia is important for both diagnosis and management. From the diagnostic viewpoint, each category encompasses a limited number of possibilities (Figure 1-3). This situation makes it possible to organize the rest of the laboratory evaluation around those tests that best discriminate among several diagnostic choices. Management of the patient will also vary according to the functional defect. For example, the need to provide a transfusion for a patient early in the course of the workup will depend on the expected ability of the patient to respond to a specific therapy (Hillman,*et al* .2005).

1.2.1.2.1 Hypoproliferative anemia

A hypoproliferative anemia (i.e. an anemia resulting from a failure in the erythroid marrow production response) can result from damage to the marrow structure or precursor stem cell pool, a lack of stimulation by erythropoietin, or iron deficiency. Patients with these conditions usually will presented with a normocytic, normochromic anemia of moderate severity. The reticulocyte production index is less than 2 and the marrow erythropoiesis: granulopoiesis ratio is less than 1:2. Measurements of red blood cell destruction indicators such as the bilirubin and lactate dehydrogenase (LDH) are normal or decreased. In essence, this is the profile of a marrow that has not responded appropriately (increased red cell production) to the patient's anemia (Hillman,*et al*. 2005).

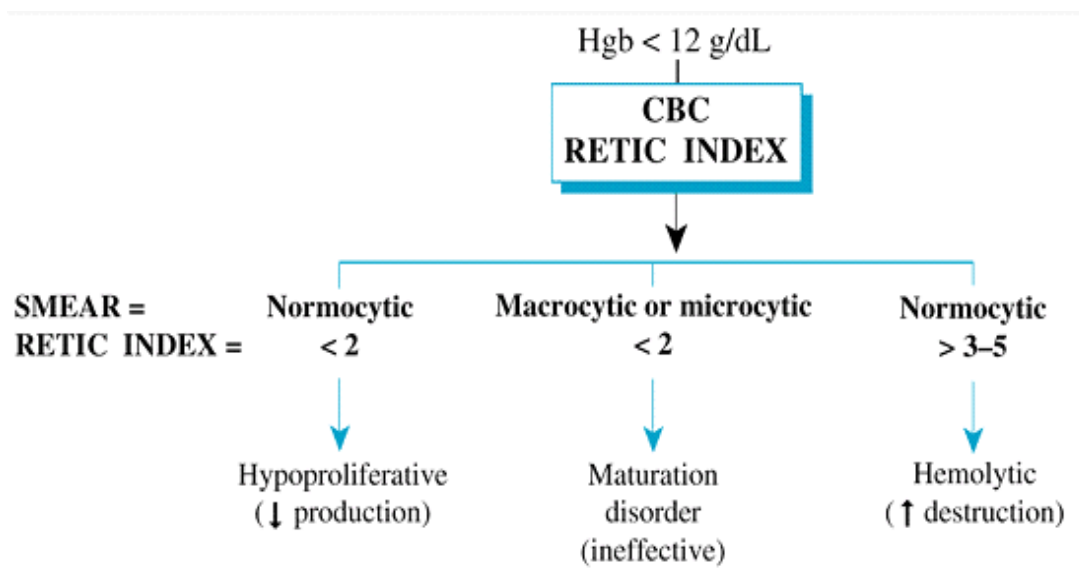


Figure (1-3): The CBC and reticulocyte index is used to classify anemia morphologically

(Adapted from Hillman,et al .2005).

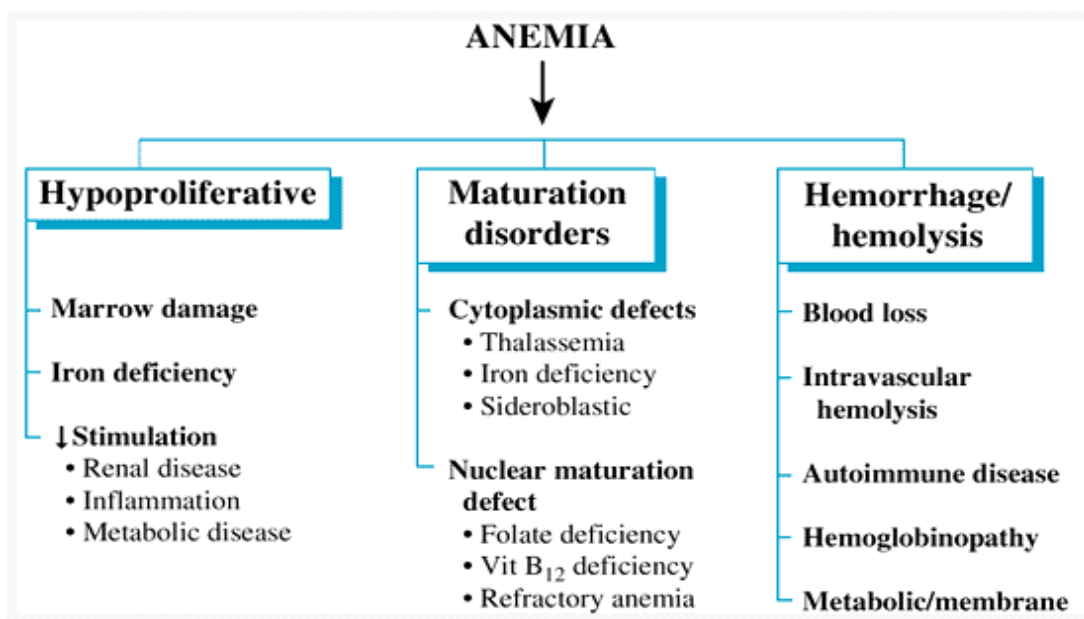


Figure (1-4): The Functional classification of anemia each of the major categories of anemia (hypoproliferative, maturation disorders, and hemorrhage/hemolysis) can be further sub classified according to the functional defect in the, several components of normal erythropoiesis (Adapted from Hillman,et al .2005).

1.2.1.2.2 Maturation Defects

Disruption of the erythroid precursor maturation sequence can result from deficiencies in vitamins such as folic acid and vitamin B₁₂, exposure to chemotherapeutic agents, or a pre-leukemic state. Since these are all defects in nuclear maturation, patients present with macrocytic anemia and megaloblastic bone marrow morphology. By contrast, defects in hemoglobinization, including severe iron deficiency and inherited defects in globin chain synthesis, the thalassemia, produce a microcytic, hypochromic anemia and markedly ineffective erythropoiesis. (Hillman,*et al* .2005).Table (1-4).

1.2.1.2.3 Increased red blood cell destruction

Blood loss or hemolysis will stimulate a compensatory red blood cell production response. Thus, the increased cell destruction category of anemia is characterized by an increase in the reticulocyte production index to greater than three times normal and a similar increase in the erythrocyte to granulocyte (E/G) ratio to levels greater than 1:1. The impact of high levels of erythropoietin stimulation in the marrow is also apparent from the appearance of large numbers of polychromatic macrocytes (marrow reticulocytes) on the peripheral blood smear. Other changes in morphology may provide a specific clue as to the cause of a hemolytic anemia (Hillman.*et al* 2005).

1.2.2 Clinical features of anemia

The symptoms and signs in an anemic patient are due to: Anemia itself and the disorder causing the anemia. The relative prominence of each of these groups of symptoms varies in the individual patient, depending on the degree of anemia and the nature and the severity of the causative disorder. Frequently, the manifestations of the causative disorder are mild or absent, and the symptoms of anemia dominate the clinical picture (Frank Firkin, *et al*. 1989).

The major adaptations to anemia are in the cardiovascular system (with increased stroke volume and tachycardia) and in the hemoglobin oxygen (O₂) dissociation curve. In some patients with quite severe anemia there may be no symptoms or signs, whereas others with mild anemia may be severely incapacitated. The presence or absence of clinical features can be considered under four major headings. the first 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 oxygen dissociation curve, secondly 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 may produce remarkably few symptoms. Thirdly age the elderly tolerate anemia less well than the young because of the effect of lack of oxygen on organs when normal cardiovascular compensation is impaired. The last landing hemoglobin oxygen dissociation curve anemia, in general, is associated with a rise in 2,3-diphosphoglycerate(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 anemia's 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(Hoffbrand,*et al.* 2006). In older subjects, symptoms of cardiac failure, angina pectoris or intermittent claudication or confusion may be present (Hoffbrand, *et al.* 2006).

1.3 Defining iron nutritional status

Iron deficiency is defined as a condition in which there are no mobilizable iron stores, resulting from a long-term negative iron balance and leading to a compromised supply of iron to the tissues (Beutler, *et al.* 2003). Iron status can be considered as a continuum: the ideal stage is normal iron status with varying amounts of stored iron within defined ranges; this is

followed by iron deficiency, characterized by the absence of measurable iron stores next, iron-deficient erythropoiesis shows evidence of a restricted iron supply in the absence of anemia; finally, the most significant negative consequence of iron deficiency (ID) is anemia, usually microcytic, hypochromic in nature (McLaren,*et al.* 1983) . Anemia in general is characterized by a decrease in number of red blood cells or less than the normal quantity of hemoglobin. The condition is determined by the expected normal range of hemoglobin in a population, and is defined as existing in an individual whose hemoglobin concentration (Hb) has fallen below a threshold lying at two standard deviations below the median for a healthy population of the same demographic characteristics, including age, sex and pregnancy status (McLean, *et al.* 1995) . Anemic conditions can result from a myriad of causes that can be isolated, but more often than not co-exist. These causes include hemolysis with malaria and other infectious diseases enzyme deficiencies, a variety of hemoglobinopathies, and other micronutrient deficiencies (McLean, *et al.* 1995). That said, the most significant contributor to the onset of anemia worldwide is iron deficiency, and thus the terms iron deficiency (ID), iron deficiency anemia(IDA), and anemia are , often falsely used interchangeably. IDA represents the most severe form of iron deficiency, and has corresponding alterations in hematological laboratory values and observable signs and symptoms. Currently, the World Health Organization accepts that generally a little less than 50% of all anemia's can be attributed to iron deficiency (McLean, *et al.* 1995).

1.3.1 Human iron metabolism

Iron is important in the formation of a number of essential compounds in the body, including but not limited to hemoglobin, myoglobin, and other metalloproteins (Lynch 1997). . Most well-nourished adults in industrialized countries contain approximately 3 to 5 grams of iron, of which about 65% is in the form of hemoglobin (Bothwell,*et al.* 1995). The remaining iron

in the body is in the form of myoglobin, other heme compounds that promote intracellular oxidization, or is stored as ferritin in the reticuloendothelial system and cells of liver hepatocytes, bone marrow, and spleen (Frazer, et al. 2005). Typically men have more stored iron than women, as women are often required to use iron stores to compensate for iron loss through menstruation, pregnancy, and lactation (Bothwell, et al. 1995).

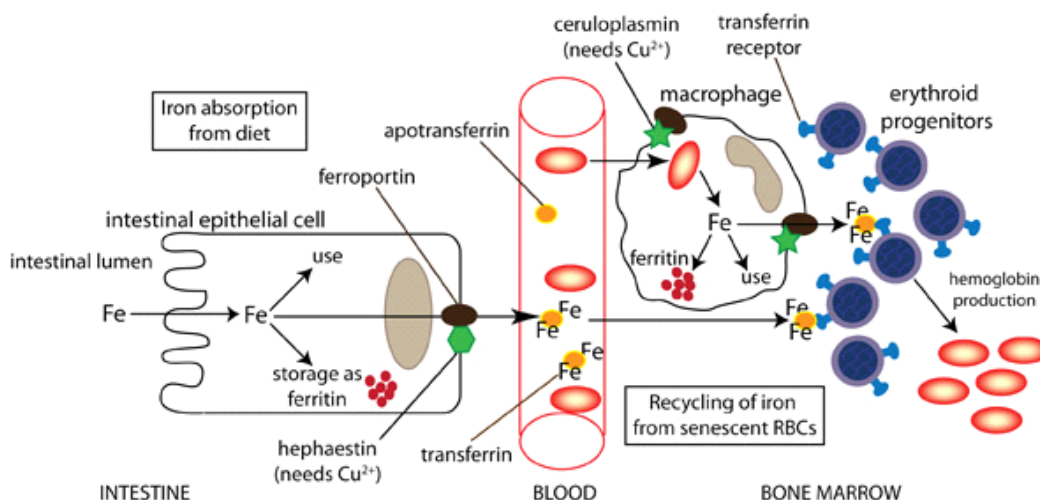


Figure 1-5: Iron metabolism (Sarika and raj kuma 2012, chapter -1)

1.3.2 Dietary iron sources and factors affect absorption

In food, two basic forms of iron exist: non-heme (inorganic) and heme (organic). (Bothwell, et al 1995). In an average diet, non-heme iron accounts for approximately 90% of total dietary iron content, while heme iron constitutes the remaining 10 %. Heme iron is highly bioavailable, and present in meat, fish, and poultry. In contrast, non heme iron is not as readily bioavailable absorption is greatly influenced by diet composition (Harvey, et al. 2000). Enhancers, such as ascorbic acid, and inhibitors, feeding habits such as phytates and polyphenols, significantly affect inorganic iron absorption (Baynes, et al. 1990). Although total iron content in a meal is an important consideration, it is crucial to appreciate that the

overall composition of the meal is of far greater significance for iron nutrition than the amount of total iron provided (McLean, *et al.* 1995) table (1-4).

Table (1-4) :Factors affecting non-haem iron absorption (Lynch SR, Dassenko SA, Cook JD *et al.* 1994).

Improved absorption	Inhibit absorption
Meat, fish and poultry	Tannins in tea, coffee and cocoa
Citrus fruit	Phytates in wholegrains
Kiwifruit	Soy protein
Tomatoes	Dietary fibre
Capsicum	
Broccoli, cauliflower	

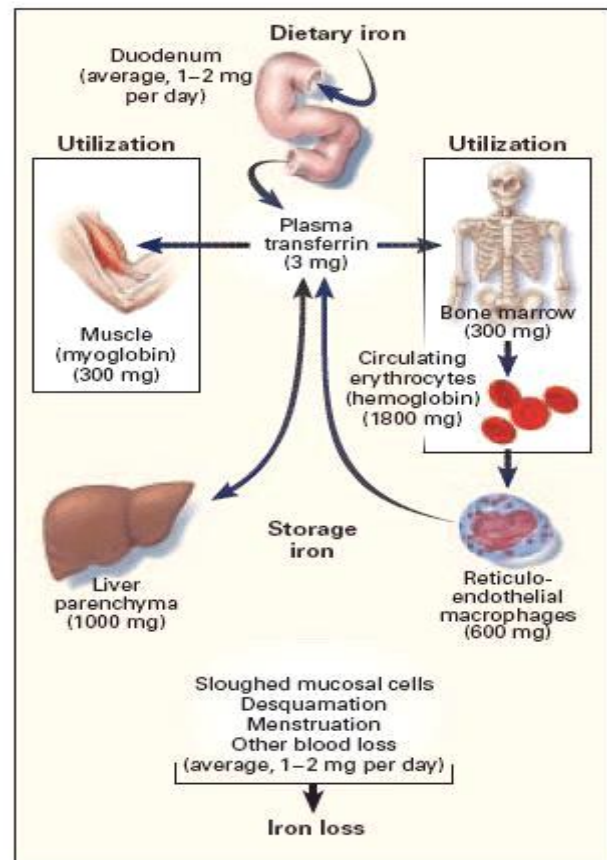
1.3.3 Mechanism of Dietary iron absorption

Dietary iron digested from food and/or supplements is absorbed by the mature villus enterocytes of the duodenum and proximal jejunum (McKie, *et al.* 2001). Non-heme and heme iron are absorbed via different pathways, though the understanding of heme iron absorption is somewhat more limited. Non-heme iron in ferrous form is transported across the apical membrane of enterocytes by a non-specific divalent metal transporter (DMT1) (Aisen, *et al.* 1999). Because much of the iron that enters the gastrointestinal tract is in the oxidized or ferric form, a duodenal ferric reductase (Dcytb) in the apical membrane of enterocytes reduces dietary iron prior to uptake (Latunde-Dada, *et al.* 2002). In contrast, heme iron molecules bind to an apical membrane protein and are absorbed intact. With the discovery of heme carrier protein 1 (HCP1), understanding has improved (Shayeghi, *et al.* 2005). HCP1 is a polypeptide belonging to a superfamily of transporter proteins, and is predicted to have nine transmembrane domains by which heme iron is taken up. Though the mechanism is unclear, research has shown that by altering gene expression in animal models, heme absorption can be enhanced or limited by overexpressing or silencing HCP1 genes, respectively. Duodenal

basolateral iron export into blood is mediated by the transmembrane protein ferroportin 1 (FPN1) (Zoller, *et al.* 2001) . The exact mechanism by which FPN1 functions is unclear, though it is thought to be facilitated by the ferroxidase activity of a membrane bound oxidase called hephaestin (Fleming, *et al.* 2005) . After moving into the plasma, iron binds to transferrin and is transported by the blood to sites of use and storage (Bailey, *et al.* 1988) . Cellular iron uptake is mediated by transferrin receptor 1 (TfR)-mediated endocytosis (Fleming, *et al.* 2005) . Once inside the cell, iron has two possible fates: incorporation into iron proteins (usually as heme) or storage as ferritin for later use during times of iron deficiency (Bleackley, *et al.* 2009) .

Figure 1-6: Distribution of iron in adult

(Adapted from American society of hematology
and anemia org.2009)



1.3.4 Regulation of iron homeostasis

Since the discovery of the hormone hepcidin in 2000, the understanding of how iron homeostasis is achieved has shifted (Bleackley, *et al.* 2009). Hepcidin a peptide hormone that is produced and predominately expressed in the liver, appears to be the master regulator of iron homeostasis in humans and other mammals (Dunn, *et al.* 2007) . When iron levels are high, several regulatory molecules including hemochromatosis gene product, hemojuvelin and transferrin receptor 2, increase hepatic hepcidin expression stimulating downstream molecular pathways. With up-regulation of hepcidin expression, iron levels are effectively regulated by binding to FPN1 which is found on the surface of iron storage cells. When iron levels are high, hepcidin causes internalization and degradation of FPN1, leading to decreased iron release from iron storage cells and a reduction in intestinal iron uptake (Viatte, *et al.* 2005). In addition, hepcidin may also play a role in negatively regulating divalent metal transporter-1 (DMT1) and duodenal cytochrome-b (Dcytb) which are involved in intestinal iron absorption; currently, the mechanism and extent of control is unknown (Viatte, *et al.* 2005). In situations where iron requirements are increased, during periods of increased erythropoietic activity, anemia and hypoxia, the down-regulation of hepcidin expression is observed, though again the mechanism is not clear (Pak,*et al.* 2006).

1.4 Hemoglobin

1.4.1 Formation of hemoglobin

Hemoglobin is an allosteric protein with primary function of binding and transporting of oxygen in the blood to tissues in order to meet metabolic demands (Baldwin, *et al.* 1979). Synthesis of Hemoglobin (Hb) involves a series of complex steps occurring in the erythrocytes, with production continuing through the early phases of the development and maturation of red blood cells (London IM, *et al.* 1964).The coordinated production of heme, the group that mediates reversible oxygenation, and globin, which is responsible for

protection of the heme group during transport, is required during synthesis (Schwartz, *et al* 1961). Fully functional hemoglobin molecules consist of four globular protein subunits, each made of a protein chain that is tightly associated with a non-protein heme group (Perutz, 1980). The first step in the synthesis of Hb is the binding of succinyl-CoA (formed during the Krebs cycle) with glycine to form a pyrrole molecule. Next, four pyrroles combine to form protoporphyrin IX, which subsequently binds with iron to form the heme molecule. Each heme molecule then combines with a ribosomal derived long polypeptide chain called a globin, forming a globular subunit of hemoglobin called a hemoglobin chain. Lastly, four Hb chains are loosely bound to produce a whole hemoglobin molecule. The most common form of Hb in adult humans, hemoglobin A, is a combination of two alpha and two beta chains arranged as a set of alpha-helix structural segments connected in a globin fold arrangement (Forget 1979). Adult and fetal hemoglobins have α chains combined with β chains (Hb A, $\alpha_2\beta_2$), δ chains (Hb A2, $\alpha_2\delta_2$) and γ chains (Hb F, $\alpha_2\gamma_2$). In embryos, α -like chains called ζ chains combine with γ chains to produce Hb Portland ($\zeta_2\gamma_2$), or with ϵ chains to make Hb Gower 1 ($\zeta_2\epsilon_2$), while α and ϵ chains form Hb Gower 2 ($\alpha_2\epsilon_2$). Fetal hemoglobin is heterogeneous; there are two varieties of γ chain that differ only in their amino acid composition at position 136, which may be occupied by either glycine or alanine; γ chains containing glycine at this position are called G γ chains, those with alanine, A γ chains.

The synthesis of hemoglobin tetramers consisting of two unlike pairs of globin chains is absolutely essential for the effective function of hemoglobin as an oxygen carrier. Figure (1-7).

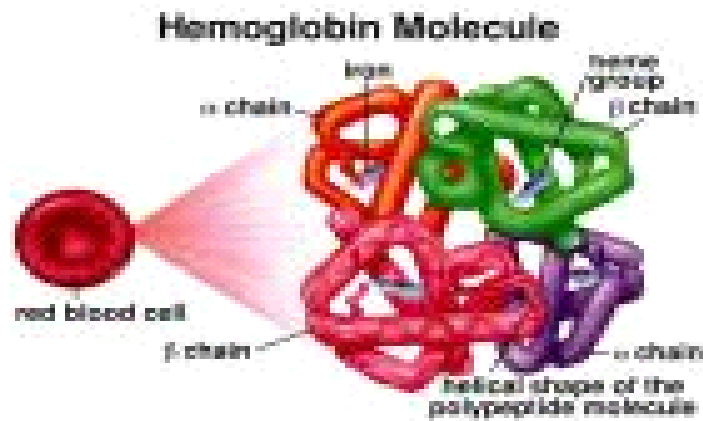


Figure 1-7: Hemoglobin Molecule (Sylvia S.mader in quiry into life eight edition).

1.4.2 Physiological control of hemoglobin levels

The primary factor regulating the production of hemoglobin is tissue oxygenation. The peptide hormone erythropoietin (EPO), responding to a feed-back mechanism measuring blood oxygenation, is synthesized in times of decreased tissue oxygenation within 24 hours of the stimulus (Faura, *et al* .1969). EPO release triggers erythrocyte production in the bone marrow in an effort to achieve homeostasis of tissue oxygenation (Fandrey 2004). As erythrocyte production increases, transferrin from plasma directly from diet and/or from iron stores enters the erythroblasts of bone marrow and is delivered to the mitochondria where heme synthesis occurs, thus inducing the formation of hemoglobin.

1.5 General introductions to Iron Deficiency anemia

Iron deficiency (ID) is defined as the decrease of the total content of iron in the body. Iron deficiency anemia (IDA) occurs when ID is sufficiently severe to reduce erythropoiesis. This type of anemia is the most frequent chronic anemia. ID may be the result of either excessive loss or, less frequently, decreased absorption. In general, the iron absorbed daily equals the amount needed to compensate its loss, so that the overall iron pool remains stable. This fine balance is easily broken, because the capability to absorb iron orally is limited. IDA occurs in 2%-5% of adult males and postmenopausal women in the developed world (Goddard , *et al*

.2000) ,(Sayer , Long 1993), with or without anemia, is even more frequent. It is a common cause of consulting a gastroenterologist (4%-13% of all referred patients) (McIntyre and Long 1993) .People whom at high risk of physiological ID, such as adolescents and pregnant women (Beutler , *et al* .2003). This strategy leads to belated evaluation, especially in young females and in patients with a prior history of anemia, resulting in significant delay in etiological diagnosis of anemia (Yates , *et al* .2004). In this sense, except in very specific situations, ID with or without anemia should always be investigated because it can be caused by potentially serious diseases (Goddard , *et al* .2000) .

1.5.1 Etiology of iron deficiency or iron deficiency anemia

There are many potential causes of ID and IDA and some of them are very relevant. Deficient intake is the most frequent etiology in the former, whereas other important diseases are potentially implicated in the case of the latter. In developed countries, the likeliest cause of anemia in each patient depends on age and sex. In women of childbearing age, excessive menstrual loss is the most frequent etiology, while in postmenopausal women and in males, digestive diseases are the main causes (McIntyre ,and Long 1993) . In addition to digestive disorders, gynecological diseases, urological diseases and other specific situations, such as intravascular hemolysis also is main causes(Table 1-4) .The etiology of ID and IDA of gastrointestinal origin can be divided into two groups: situations with increased loss of iron (the most common in developed countries), and those with decreased Iron absorption. In the former, the blood loss can occur in the form of visible bleeding (melena, hematemesis, rectal bleeding) or hidden bleeding . benign or malignant gastrointestinal tumors of the colon, stomach, esophagus and small intestine, peptic ulcer and reflux disease, use of non-steroidal anti-inflammatory drugs (NSAIDs), and inflammatory bowel disease. Reduced iron absorption is the second category of ID causes of digestive origin, and can be caused by celiac

disease, atrophic gastritis, and postsurgical status (gastrostomy, intestinal resection) among others. Celiac. Disease is very relevant and specific evaluation to exclude it must be performed. The prevalence of this disease worldwide is approximately 1%, and it is probably under diagnosed (Catassi , *et al* .2008).

Table 1-4: Causes of iron deficiency anemia (Adapted From World Journal of Gastroenterology 2009).

• Digestive disorders
• Increased losses of iron
• Cancer/polyp: colon, stomach, esophagus, small bowel
• Peptic ulcer, esophagitis
• NSAID use
• Inflammatory bowel disease: ulcerative colitis, Crohn's disease
• Intestinal parasites
• Vascular lesions: angiodysplasia, watermelon stomach
• Meckel's diverticulum
• Reduced iron absorption
• Celiac disease - Whipple's disease
• Bacterial overgrowth
• Lymphangiectasia
• Gastrectomy (partial and total) and gastric atrophy
• Gut resection or bypass
• Urological and gynecological disorders
• Intravascular hemolysis
• Prosthetic valves and cardiac myxomas, paroxysmal nocturnal hemoglobinuria, marathon runners, multiple blood donations
• Deficient iron intake

1.5.2 Anemia in pregnancy

Anemia in pregnancy is defined as an Hb<110g/l in first and last trimester. and a Hb<105g/L in the second trimester (Centers for Disease Control and Prevention 1998). Women with anemia in pregnancy may experience fatigue, reduced energy levels, reduced mental performances (Breyman C, Bian X, *et al* 2010), and in cases of severe anemia it is associated with preterm birth, low birth weights, and a small for gestational age fetus. In the postpartum period anemia has been found to be linked to depression, emotional instability, stress and lower cognitive performance tests (Milman N. 2008).

The most common causes of anemia in pregnancy include iron deficiency, folate deficiency vitamin B12 deficiency, hemolytic diseases, bone marrow suppression, chronic blood loss and underlying malignancies (Reveiz L, Gyte GMI, Cuervo LG 2007). 30-50% of women become anemic during pregnancy, with iron deficiency being the most common form of anemia in more than 90% of the cases (Johnson TA. Anemia 2010).

The gastrointestinal tract increases iron absorption when the body's iron stores are low, and it reduces the absorption when there are sufficient stores. Requirement for absorbed iron ranges from 0.8mg/day in the first trimester to 7.5 mg/day in the second trimester, averaging approximately 4.4 mg/day in pregnancy (Pavord S, Myers *et al* 2012). Iron requirements increase rapidly in the second and third trimester due to fetal growth, however iron absorption in the gut is not sufficient to meet this increased demand. Thus iron balance depends on maternal iron stores during this period (Johnson TA. Anemia 2010).

A trial of oral iron should be considered as a diagnostic test for all pregnant women with suspected iron deficiency anemia (IDA). The hemoglobin should increase within 2 weeks, otherwise further tests are required (Pavord S, Myers *et al* 2012). Oral iron supplementation is the primary treatment option.

1.6. Rationale

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children.

Iron deficiency is the most prevalent nutritional deficiency and the major cause of anemia worldwide. The World Health Organization (WHO) estimates that iron deficiency is responsible for approximately 50% of all anemia cases. In women of childbearing age, the anemia prevalence is 30.2%; Overall, 468.4 million women of childbearing age are anemic. In Saudi Arabia most of the studies on anemia were based on nutritional status and concentrated on preschool children who were under six years old (Sebai et al., 1981; Serenius and Fourgerson, 1981; Sebai, 1988; Al-Othaimeen et al., 1988; Rasheed et al., 1989), so data on the nutritional status of children and adolescents in the Kingdom are insufficient (El-Hazmi and Warsy, 1999; Abalkhail and Shawky, 2002).

There is no published study was done to detect and evaluate the type of anemia among women in El-Khurmah province western Saudi Arabia , despite the spread of anemia in this province.

1.7 Objectives

1.7.1 General Objective

To determine the frequency of anemia in pregnant and non- pregnant Saudi women in EL-Khormah Province Western Kingdom of Saudi Arabia.

1.7.2 Specific Objectives

1. To detect the anemia in pregnant and non-pregnant Saudi women that was attending to Al-Elaj Medical Clinic in El-Khormah province.
2. To evaluate the iron status related measurements in the study groups.
3. To evaluate RBCs morphology on blood film of study groups.
4. To find hemoglobin types by electrophoresis to differentiate iron deficiency anemia from thalassemia minor.

Chapter II

2. Materials and Methods

2.1 Study design

Analytical, descriptive and cross-sectional study was conducted to determine the Type and frequency of anemia among pregnant and non-pregnant Saudi women in El-Khormah Province Western area of kingdom of Saudi Arabia during the period August 2012 to August 2014.

2.2 Study area

The study was conducted in El-khuraamh Province Western of kingdom Saudi Arabia .

2.3 Study population

Pregnant and non-pregnant Saudi women at reproductive age in El-khormah province Western of kingdom Saudi Arabia

2-3-1 Sampling

Five ml of venous blood were collected under aseptic condition. 2.5 ml of venous blood in ethylene-diamine-tetra-acetic acid (EDTA) anticoagulated vacutainer tubes to perform a complete blood count (CBC), thin blood film, reticulocyte count and hemoglobin electrophoresis. 2.5 ml of venous blood was collected in vacutainer gel tubes to perform iron profile .The study sample size was set as six hundred (600) participants.

2-3-2 Inclusion criteria

Pregnant and non-pregnant Saudi women in reproductive age .

2.3.3 Exclusion criteria

Co-operative pregnant women , those < 18 years and those with history of diabetes mellitus, hypertension , those on iron supplements(for non- pregnant women participants), history of blood transfusion within the last 3 months, and those with any inflammatory disease .

2.3.4 Sample size

Three hundred of pregnant women (one hundred fifty with prophylactic iron therapy and one hundred fifty without prophylactic iron therapy) , two hundred and fifty of non-pregnant women and fifty were control participants were included consenting apparently healthy, non- pregnant, non- menopausal and non- menstruating(at the time of recruitment and sample collection) all groups at reproductive age .

2.3.5 Data and Questionnaire Information

The data was collected using laboratory investigations .the questionnaire for collecting the information of age, kidney disease, diabetes, history of blood transfusion last 3 month, history of hypertension, medical prenatal care started, craving or chewing ice and etc.

2.4 Methodology

2.4.1 Method of sample collection

- **Requirements**

Ethylenediaminetetraacetic acid (EDTA) anticoagulated vacutainer tubes and vacutainer gel tubes containers (EBOS. FACTORY), cotton, alcohol (70%), syringes and tourniquet.

Five ml of venous blood was collected under aseptic condition. 2.5 ml of venous blood in ethylene-diamine-tetra-acetic acid (EDTA) anticoagulated vacutainer tubes to perform a complete blood count (CBC), thin blood film, Reticulocyte count and Hemoglobin electrophoresis. 2.5 ml of venous blood was collected in to vacutainer gel tubes to perform iron profile

- **Procedure**

Patient was either set or lie up right on an examination table then the arm was positioned on the armrest so that the vein identified is under some tension and its mobility was reduced after that the skin was cleaned with 70% ethanol and allowed to dry, to avoid stinging when the skin is penetrated. A tourniquet was applied to the arm, sufficiently tightly to distend the vein,

but not so tightly that discomfort was caused then Personal details was checked up on the forms and blood vials, after that two and have ml of blood samples was taken from the inside of the upper arm median cubital vein. Then blood was collected in EDTA (1.2 mg/ml blood) anticoagulant. (Lewis Mitchell, *et al.* 2006).

2.4.2 Method of automated analyzer system (Complete hemogram)

Particles can be counted and sized either by electrical impedance or by light scattering. Automated instruments have at least two channels. In one channel a diluent is added and red cells are counted and sized. In another channel a lytic agent is added, together with diluent, to reduce red cells to stroma, leaving the white cells intact for counting and also producing a solution in which Hb can be measured. Further channels are required for a differential WBC, which is often dependent on study of cells by a number of modalities, e.g. impedance technology with current of various frequencies, light scattering and light absorbance. A separate channel or an independent instrument may be required for a reticulocyte count.

- **Requirements**

Cell Dyn 1700, automated blood cells count. Commercially blood cells control (High control, Normal control, and Low control).

- **Detection Principles**

Direct current (DC) detection method (WBC, RBC, PLT).

Non-cyanide method (HGB).

Cumulative pulse height detection method (HCT).

- **Procedure**

The reagent needed was checked then the power switch was turned on self-auto rinse, and background check was automatically performed and the vend (vend for analysis) was appeared, after that whole blood mode was selected. Sample number: inputted by pressing

sample number then number of sample was entered, then enter key was pressed. Sample was mixed sufficiently. The tube was set to the sample probe, and in that, condition the start switch was pressed. When the liquid-crystal display (LCD) screen display analyzing the tube was removed, after that, the unit executes automatic analysis and the result was displayed in the screen, then the result was printed out.(Cell dyn 1700 Abbott 2010) .

2.4.3 Method of Preparation and staining of blood films

- **Requirements**

Slides (76 × 26 mm and approximately 1.2 mm thick) (CITOTEST LABWARE MFG.CO.LTD. CHINA), spreader (from a glass slide that has a smooth end), absolute methanol and Romanowsky stains (Leishman's stain).

- **Procedure**

Blood films were prepared from fresh blood with no anticoagulant added or from ethylene diamine tetra –acetic acid (EDTA) anticoagulant.

A small drop of blood was placed in the center line of slide about 1 cm from one end, then without delay a spreader was placed in front of the drop at an angle of about 30 degree to the slide and move it back to make contact with the drop, the drop must spread out quickly along the line of contact. With a steady movement of the hand, the drop of the blood was sprit along the slide, after that the films was allowed to dry in the air, then the film was labeled immediately after spreading, then the films were fixed by absolute methanol, then the film was placed on a staining rack, flood with leishman's stain, and left for 2 minutes to be fixed twice buffered distilled water was added and left to stain for 10 min, then stain was washed off with tap water. After the back of the slide has been wiped clean, set it up right to dry, then the film was examined macroscopically to assess whether the spreading technique was

satisfactory and to judge its staining characteristic and whether there are any abnormal particles present.

2.4.4 Reticulocyte count

- **Requirements**

Test tube 12x75 mm, droppers and methylene blue stain.

- **Procedure**

Reticulocytes count performed after mixing equal volume of blood collected in EDTA with methylene blue and thin blood film made. After drying, the film examined by oil immersion lens to assess reticulocyte count after certain calculations.

2.4.5 Measurement of Serum Iron (SI)

Serum iron was measured using photoelectric colorimeter Ap-101 in the Clinical Chemistry laboratory at Al-Elaj clinic. Commercial kits available for (SI). Precise and accuracy of all methods were checked by commercially prepared control sample before the application of test and control samples.

- **Method**

Iron (II) react with chromazurol B (CAB) and cetyltrimethylammoniumbromide(CTMA) To form colored ternary complex with an absorbance maximum at 623nm.the intensity proportional to the concentration of iron in the sample.

2.4.6 Measurement of Serum ferritin

Serum ferritin will be measured using photoelectric colorimeter Ap-101 in the clinical Chemistry laboratory at Al-Elaj clinic, commercial kits available for serum ferritin. Precise and accuracy of all methods were checked by commercially prepared control sample before the application of test and control samples.

- **Method**

Ferritin in sample or standard cause agglutination of latex particles coated with anti-ferritin antibodies .the agglutination is proportional to the ferritin concentration in sample or standard and can be measured by turbidimetry.

2.4.7 Measurement of total iron binding capacity (TIBC)

Total iron binding capacity (TIBC) was measured using photoelectric colorimeter Ap-101 in the clinical Chemistry laboratory at Al-Elaj clinic, commercial kits available for Total iron binding capacity. Precise and accuracy of all methods were checked by commercially prepared control sample before the application of test and control samples.

- **Method**

The iron binding protein transferring in serum is saturated upon treatment with an excess of iron $Fe(III)$ ions .unbound (excess) iron is adsorbed onto aluminum oxide and precipitated. The transferring bound iron (TIBC) in the supernatant is the determined.

2.4.8 Measurement of Unsaturated iron binding capacity (UIBC)

For the calculation of the latent or unsaturated binding capacity the serum iron is subtracted from the TIBC.

2.4.9 Electrophoresis method

- **Requirements**

Electrophoresis tank and power pack. Any horizontal electrophoresis tank that will allow a bridge gap of 7 cm. A direct current power supply capable of delivering 350 V at 50 mA is suitable for both cellulose acetate and citrate agar electrophoresis, wicks of filter or chromatography paper, blotting paper, cellulose acetate membranes. Plastic-backed membranes (7.6×6.0 cm) are recommended for ease of use and storage, staining equipment, electrophoresis buffer. Tris/EDTA/borate (TEB) pH 8.5. Tris-(hydroxymethyl) aminomethane

(Tris), 10.2 g, EDTA (disodium salt), 0.6 g, boric acid, 3.2 g, water to 1 litre. The buffer should be stored at 4°C and can be used up to 10 times without deterioration, wetting agent. For example, Zip-prep solution (Helena Laboratories).

- **Principle**

At alkaline pH, hemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode (+). Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from Hb A. Hemoglobin variants that have an amino acid substitution that is internally sited may not separate, and those that have an amino acid substitution that has no effect on overall charge will not separate by electrophoresis. (Lewis Mitchell, *et al.* (2006). Practical Hematology. 10th edition. Philadelphia, United States of America. Elsevier Ltd).

- **Procedure**

Samples were centrifuged at 1200 g for 5 min. Then twenty (20) µl of the packed red cells was diluted with 150 µl of the haemolyzing reagent and was mixed gently and left for at least 5 min, the electrophoresis tank was prepared by placing equal amounts of tris/borate/edta(TEB) buffer in each of the outer buffer compartments. Wet two chamber wicks in the buffer, and placed one along each divider/bridge support ensuring that they make good contact with the buffer after that the cellulose acetate was soaked at least 15 min before used, then the sample well plate was filled with 5 µl of each diluted sample or control and was covered with a 50-mm cover slip or a “short” glass slide to prevent evaporation. a second sample in well plate was loaded with Zip-prep solution, then the applicator tips immediately prior was cleaned to use by loading with Zip-prep solution and then applying them to a blotter, after that the cellulose acetate strip was removed from the buffer and blotted twice between two layers of clean blotting paper. The cellulose acetate was not allowed to dry, and

then the applicator was loaded by depressing the tips into the sample wells twice, and applied this first loading onto some clean blotting paper. The applicator was reloaded and applied the samples to the cellulose acetate. Then the cellulose acetate plates were placed across the bridges, with the plastic side uppermost. Two glass slides were placed across the strip to maintain good contact. After 25 min electrophoresis at 350 V, immediately the cellulose acetate was transferred to Ponceau S and fixed and stained for 5 min, after that excess stain was removed by washing for 5 min in the first acetic acid reservoir and for 10 min in each of the remaining two. Blot and left to dry, then the membranes was labeled and stored in a protective plastic envelope.

2.5 Quality Control Method

Precise and accuracy of all methods were checked by commercially prepared control sample before the application of test and control samples.

2.6 Ethical consideration

Permission for this study was obtained from local health authorities in the area of the study and also from Sudan university of science and technology (SUST), An informed consent was obtained from all participants

2.7 Statistical analysis:

Computer software of Statistical Package for Social Science (SPSS) was used for data analysis. The mean, correlations and standard deviation of the hematological parameters and iron profile were calculated and student *t*-test was used for comparison. Independent sample T-test was used to calculate P value. The P-value of less than 0.05 was considered statistically significant.

Chapter III

3. Results

3.1 General characteristics

The mean age among non-pregnant women were 33.2 ± 4.3 years ,table (3-1) , the majority of them in the age group of 26-35 years (50.4 %) as shown in table (3-1), and the mean age among pregnant women were 29.6 ± 4.4 years, table (3-1) , majority of them in the age of 26-35 years(71.0%) as shown in table (3-1).

Table (3-1): The age groups and std. deviation among pregnant and non-pregnant women.

The age groups among non-pregnant women	frequency	Percent %	Mean \pm SD	The age groups among pregnant women	frequency	Percent %	Mean \pm SD
18-25 years	59	23.6	33.2 ± 4.3	18-25 years	56	18.7	29.6 ± 4.48
26-35 years	126	50.4		26-35 years	213	71	
36-45 years	65	26		36-45 years	31	10.3	
Total	250	100		Total	300	100	
Total							

3.2 Laboratory investigations

The mean of hematological parameters of control groups was compared with that of non-pregnant women, pregnant women with iron therapy, and pregnant women without iron therapy as shown in table (3-2).it was all increased in control group against others groups except in the mean of total erythrocyte count there was no significant difference between control group, non-pregnant women, and pregnant women with iron therapy. Also the mean of reticulocyte count decreased in control group against that of pregnant women with iron therapy and same mean in pregnant women without iron therapy.

The hematological parameters among non-pregnant women was compared with that of pregnant women with prophylactic iron therapy and pregnant women without prophylactic

iron therapy as shown in table (3-3), the mean hemoglobin level (Hb) , hematocrit (Hct) was increased in non-pregnant women compared with pregnant women with prophylactic iron therapy ($p < 0.061$), ($p < 0.077$) respectively , but there was no significant difference in the mean of total erythrocyte count (RBC) , mean cell volume (MCV), and mean cell hemoglobin concentration(MCHC) ($p < 0.055$), ($p < 0.051$) , ($p < 0.053$) respectively .

The mean of red mean hemoglobin concentration (MCH) and reticulocyte count in pregnant women with prophylactic iron therapy was increased than that of non-pregnant women ($p < 0.06$) ($p < 0.004$) respectively. In other side the mean of (RBC), (Hb), (Hct) ,(MCV) ,(MCH) , (MCHC) and reticulocyte count was decreased in pregnant women without iron therapy when compared with that of non-pregnant women and pregnant women with prophylactic iron therapy ($p < 0.065$) ($p < 0.055$) , ($p < 0.06$) ($p < 0.001$), ($p < 0.006$) (0.07), ($p < 0.004$) (0.005) ,($p < 0.007$) (0.005), (0.053) (0.05) and (0.0)(0.001) respectively ,table (3-3).

Blood cells distribution width (RDW) mean was increased in pregnant women without prophylactic iron therapy against other study groups table(3-2), and the mean of reticulocyte count was increased in pregnant women with prophylactic iron therapy when compared with that of non-pregnant women and pregnant women without prophylactic iron therapy ($p < 0.004$)($p < 0.001$) respectively (table 3-3,3-4).

Blood film examination indicated that (73.2%) , (68.6%) and (78.6%) of non-pregnant women ,pregnant women with prophylactic iron therapy , and pregnant women without prophylactic iron therapy respectively, that met the WHO definition of anemia (Hb < 12 g/dl , < 11 g/dL) showed a microcytic hypochromic red cell picture figure (3-1).

The biochemical results including serum iron, serum ferritin, and TS%, was increased in control group when compared with other groups, but we noted that TIBC% was decreased table (3-2). It was showed variable results in other study group , the mean of serum iron of

non-pregnant women was increased than that of pregnant women with prophylactic iron therapy and pregnant women without prophylactic iron therapy ($p<0.001$), ($p<0.006$) respectively. Table (3-3, 3-4). And it was increased in pregnant women with prophylactic iron therapy when compared with that of pregnant women without iron therapy ($p<0.065$). The mean of total iron binding capacity (TIBC) showed no significant difference in subjects groups. ($p<0.05$), ($p<0.07$) and ($p<0.07$) respectively figure (3-5). The mean of transferrin saturation (TS %) in non-pregnant women groups was increased when compared with that of pregnant women with prophylactic iron therapy group ($p<0.001$), ($p<0.005$) respectively, table (3-3). And TS% of pregnant women with prophylactic iron therapy was high than that of pregnant women without prophylactic iron therapy ($p<0.058$) table (3-5).

it was noted that the mean of serum ferritin level in non-pregnant women was increased than that of pregnant women with and without prophylactic iron therapy ($p<0.004$) ($p<0.001$) respectively ,table (3-3, 3-4). In same time the mean of serum ferritin level in pregnant women with prophylactic iron therapy was increased than that of pregnant women without prophylactic iron therapy ($p<0.05$) table (3-5) . The percentage of anemia and IDA in first trimester in pregnant women with prophylactic iron therapy was 4.7 %, 4.55 %, and 5.13 % respectively table (3-7).. In second trimester the prevalence was 36.47 %, 45.45% and 46.1 % respectively table (3-7). The percentage were 58.8 %, 50 % ,48.7 % respectively in third trimester and total of subject in this period were (59) subjects.

As for spacing, the highest prevalence was found among pregnant women who had spacing of less than one year and up to one year (43, 3%). The lowest prevalence was observed among those whose spacing was more than two years (24.6 %) table (3-9).

Table (3-2): The mean of hematological and biochemical parameters in normal group (Control), non-pregnant women, pregnant women with and without iron therapy.

Parameter	Control	Non pregnant group	Pregnant group with iron therapy	Pregnant group without iron therapy
	Mean \pm SD			
Total erythrocytes ($10^6/\mu\text{l}$)	4.28 \pm 0.25	4.18 \pm 0.35	4.21 \pm 0.353	3.81 \pm 0.48
Hemoglobin (g/dl)	12.97 \pm 0.4	11.8 \pm 1.02	10.9 \pm 1.2	9.8 \pm 0.99
Hematocrit (Hct) %	38.8 \pm 1.4	35.4 \pm 3.9	33.3 \pm 2.9	29.9 \pm 3.5
Mean cell volume (MCV) fl	90.7 \pm 5.2	83.2 \pm 9.2	83.1 \pm 7.5	75.4 \pm 8.6
Mean cell hemoglobin (MCH) pg	30.2 \pm 1.6	27.8 \pm 3.1	31.2 \pm 2.6	24.3 \pm 3.2
Mean cell hemoglobin concentration (MCHC) g/dl	33.4 \pm 0.47	31.6 \pm 2.5	31.1 \pm 2.1	24.3 \pm 3.2
Reticulocyte %	1.1 \pm 0.11	1.1 \pm 0.1	2.5 \pm 1.7	0.5 \pm 0.3
RDW %	12.5 \pm 0.82	14 \pm 0.73	15.3 \pm 1.71	16.2 \pm 2.67
Hb electrophoresis	HbA	HbA	HbA	HbA
S. Iron ($\mu\text{g/dl}$)	91.9 \pm 8.6	80 \pm 14.40	68.7 \pm 15.60	61.1 \pm 7.53
S. Ferritin (ng/ml)	49.5 \pm 9.0	19.5 \pm 9.30	14.08 \pm 2.683	10.53 \pm 1.945
TIBC ($\mu\text{g/dl}$)	302 \pm 14.5	325 \pm 53.60	370 \pm 21.90	377 \pm 35.90
TS %	30 \pm 2.6	25.2 \pm 6.114	18.6 \pm 4.444	16.36 \pm 2.889

Table (3-3): The mean of hematological, biochemical parameters and retics count in non-pregnant and pregnant women with prophylactic iron therapy group.

The parameters	Non-preg.	Preg. With iron	P. value
	Mean±SD		
Total erythrocytes (10 ⁶ / μl)	4.18±0.35	4.21±3.53	0.055
Hemoglobin (g/dl)	11.8±1.02	10.9±1.2	0.061
Packed cell volume (PCV) or Hematocrit (Hct) %	35.4±3.9	33.3±2.9	0.077
Mean cell volume (MCV) fl	83.2±9.2	83.1±7.5	0.051
Mean cell hemoglobin concentration (MCHC) g/dl	31.6±2.5	31.1±2.1	0.053
Mean cell hemoglobin (MCH) pg	27.8±3.1	31.2±2.6	0.060
RDW %	14±0.73	15.3±1.71	0.081
Retics count	1.1±0.1	2.5±1.7	0.004
S. Iron (μg/dl)	80.0±14.4	68.7±15.6	0.001
S. Ferritin (ng/ml)	19.5±9.3	14.08±2.6	0.004
TIBC (μg/dl)	325±53.6	370±21.9	0.050
TS %	25.2±6.114	18.6±4.444	0.001

Table (3-4): The mean of hematological, biochemical parameters and retics count in non-pregnant and pregnant women without prophylactic iron therapy group

The parameters	Non-preg.	Preg. Without iron	P. value
	Mean±SD		
Total erythrocytes (10 ⁶ / µl)	4.18±0.35	3.81±0.48	0.065
Hemoglobin (g/dl)	11.8±1.02	9.8±0.99	0.001
Packed cell volume (PCV) or Hematocrit (Hct) %	35.4±3.9	29.9±3.5	0.006
Mean cell volume (MCV) fl	83.2±9.2	75.4±8.6	0.004
Mean cell hemoglobin concentration (MCHC) g/dl	31.6±2.5	24.3±3.2	0.007
Mean cell hemoglobin (MCH) pg	27.8±3.1	24.3±3.2	0.050
Retics count	1.1±0.1	0.5±0.3	0.0
RDW%	14±0.73	16.2±2.67	0.007
S. Iron (µg/dl)	80.0±14.4	61.1±7.53	0.006
S. Ferritin (ng/ml)	19.5±9.3	10.53±1.94	0.001
TIBC (µg/dl)	325±53.6	377±35.9	0.07
TS %	25.2±6.114	16.36±2.889	0.005

Table (3-5): The mean of hematological, biochemical parameters and retics count pregnant women with prophylactic iron therapy and pregnant women without prophylactic iron therapy group.

The parameters	Preg. With iron	Preg. Without iron	P. value
	Mean \pm SD		
Total erythrocytes (10 ⁶ / μ l)	4.21 \pm 3.53	3.81 \pm 0.48	0.055
Hemoglobin (g/dl)	10.9 \pm 1.2	9.8 \pm 0.99	0.061
Packed cell volume (PCV) or Hematocrit (Hct) %	33.3 \pm 2.9	29.9 \pm 3.5	0.077
Mean cell volume (MCV) fl	83.1 \pm 7.5	75.4 \pm 8.6	0.005
Mean cell hemoglobin concentration (MCHC) g/dl	31.1 \pm 2.1	24.3 \pm 3.2	0.053
Mean cell hemoglobin (MCH) pg	31.2 \pm 2.6	24.3 \pm 3.2	0.050
RDW%	15.3 \pm 1.71	16.2 \pm 2.67	0.068
Retics count	2.5 \pm 1.7	0.5 \pm 0.3	0.001
S. Iron (μ g/dl)	68.7 \pm 15.6	61.1 \pm 7.53	0.065
S. Ferritin (ng/ml)	14.08 \pm 2.6	10.53 \pm 1.94	0.054
TIBC (μ g/dl)	370 \pm 21.9	377 \pm 35.90	0.070
TS %	18.6 \pm 4.444	16.36 \pm 2.88	0.058

Table (3-6): The percentage of anemia and iron deficiency anemia according to the WHO criteria in non-pregnant, pregnant with and without prophylactic iron therapy.

anemia with diagnostic Criteria	Non-pregnant women N (%)	Pregnant with iron therapy N (%)	Pregnant without iron therapy N (%)
Hemoglobin < 11 g/dl (Hb <12 g/dl for non-pregnant groups)	118 (47.2 %)	85 (56.6 %)	116 (77.3 %)
S. Ferritin < 12 ng/ml	42 (16.8 %)	44 (29.3 %)	94 (62.6 %)
Transferrin saturation <16 %	20 (8 %)	39 (26 %)	51 (34 %)

Table (3-7): Percentage of anemia and iron deficiency anemia based on trimester in pregnant women with iron therapy.

Trimester of pregnancy	Number of subject	Number (%) with anemia Hb< 11 g/dl	Number (%) with IDA ferritin < 12 ng/ml	Number (%) with TS < 16 %
First	10	4 (4.70 %)	2 (4.55 %)	2 (5.13 %)
Second	81	31 (36.47 %)	20 (45.45%)	18 (46.15%)
Third	59	50 (58.8 2%)	22 (50.0%)	19 (48.71%)

Table (3-8) : Percentage of anemia and iron deficiency anemia based on trimester in pregnant women without iron therapy.

Trimester of pregnancy	Number of subject	Number (%) with anemia Hb< 11 g/dl	Number (%) with IDA ferritin < 12 ng/ml	Number (%) with TS < 16 %
First	61	37 (31.50 %)	20 (21.30 %)	11 (21.57 %)
Second	49	39 (33.20 %)	36 (38.3%)	20 (39.21%)
Third	40	40 (35.30%)	39 (39.4%)	20 (39.21%)

Table (3-9) : Space between pregnancy in pregnant women .

Less than one year up to one year	43, 3%
More than one year up to two years	24.6 %

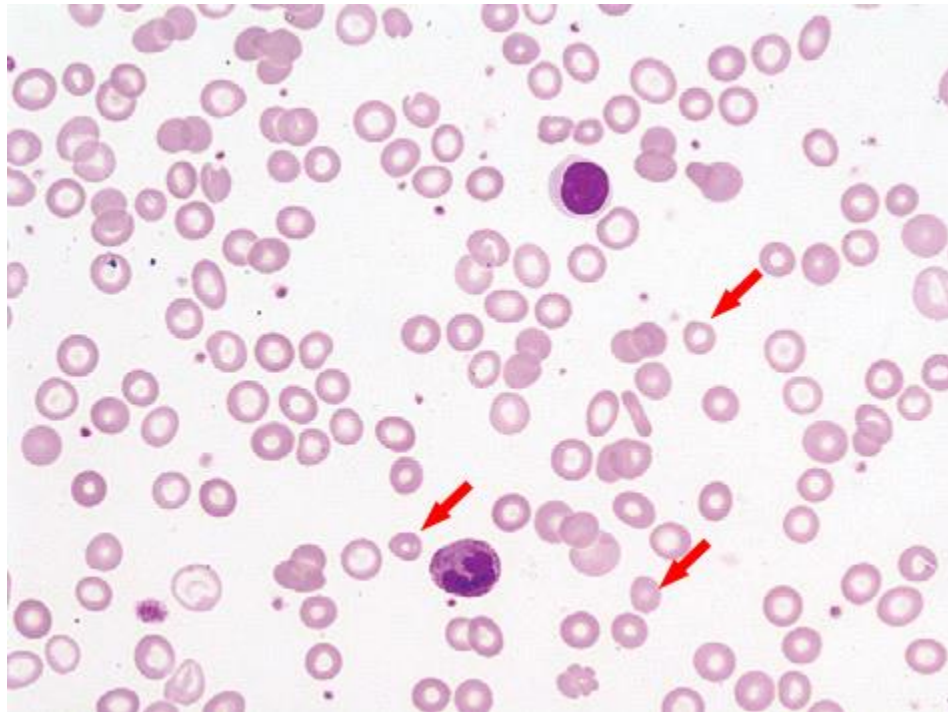


Figure (3-1): Blood film from one of the subject with IDA showing Microcytic hypochromic red blood cells.

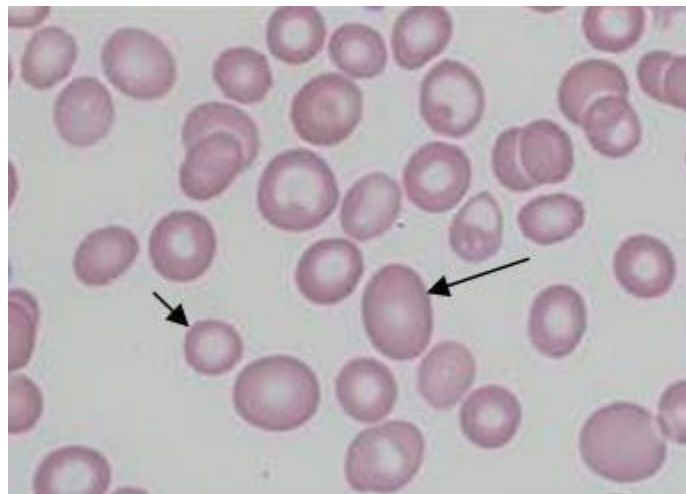


Figure (3-2): Dimorphic blood picture from thin blood film of one of the subject with IDA on iron supplementation showing microcytic hypochromic red blood cell mixed with normocytic normochromic red blood cells.

Chapter IV

4. Discussion, conclusion and recommendations

4.1 Discussion

Iron deficiency is the most common form of nutritional anemia worldwide and was estimated to affect 1.3 to 2.2 billion persons worldwide. Justification for the screening of pregnant women for iron deficiency anemia has been based on an association of anemia with an increased risk for preterm delivery and other complications.

The results showed that the most type of anemia in all study groups was iron deficiency anemia. The percentage of anemia based on WHO criteria ($Hb < 12g/dl$) in non-pregnant women and ($Hb < 11 g/dl$) in pregnant women , Ferritin ($< 12.0 ng/ml$) and transferrin saturation ($TS < 16 \%$) was higher in pregnant women without iron therapy than other groups (77.3 %) and it was higher than that percentage recorded in Jeddah Saudi Arabia (25.6%) (Ghaznawi, *et al* .1989) .

Reticulocytosis associated with anemia was noted in pregnant women with prophylactic therapy and this was suggested that iron is the fuel for the production of new red blood cells (Adamson, JW 1994). The evidence of dimorphic red blood cells picture was noted in blood films of pregnant women with iron therapy figure(3-2) and it was consistent with previous report which indicated that up to 40% of patient with true IDA have normocytic normochromic red blood cells.(Bermejo , et al 2009). Poikilocytosis, anisocytosis, pencil-shaped cells and few nucleated red blood cells were markedly shown in pregnant women without iron therapy thin blood film. Platelets is slightly increased in all groups (mild thrombocytosis). No abnormal hemoglobin pattern was found in our study group. (All groups were adult Hemoglobin pattern ,no HbA_2 not found).

The diagnosis of IDA using iron related parameter is associated with a number of challenges. Serum iron levels are not helpful by themselves because they vary with time of the day and due to various systemic insults (Favier , *et al* 1983). Another challenge is that although serum iron levels can be measured directly in the blood, but these levels increase immediately with iron supplementation (in this study subjects stopped supplementation for 24 hours) and pure blood serum iron concentration in any case is not as sensitive as a combination of total serum iron along with a measure of the serum iron-binding protein levels-(total iron binding capacity TIBC). The ratio of serum iron to TIBC (transferrin saturation index or percent) is the most specific indicator of iron deficiency, when it is sufficiently low .So the transferrin saturation index of non-pregnant group was higher when compared with that of pregnant groups , and transferrin saturation index of pregnant women with iron therapy in this study were increased than that of pregnant women without iron therapy .

The percentage of anemia based on WHO criteria ($Hb < 12g/dl$) among non-pregnant subjects group was (47.2%) , our finding agree with the previous reports of health nutritional and population statistics , 47.9 % was recorded at 1995 in Saudi Arabia. There are several factors that may be responsible for the higher percentage of IDA among non-pregnant women in this study. El-khurmah is a province in western Saudi Arabia, like in other developing countries there is high incidence of malnutrition with resultant nutritional anemia despite the higher economic income of the families per month .daily food lacks the essential amount of iron and vitamins, most ladies chewing ice especially schoolgirls and university students, most of women skip breakfast meal and drinking a lot of coffee.

The percentage of anemia and IDA in first trimester in pregnant women with prophylactic iron therapy according to WHO Definition was low when it compare with that of second and

third trimester . The total number of pregnant women with prophylactic iron therapy was low in first trimester (10 subject) and this is due to the increase nausea when subjects taking iron therapy during first trimester. and the percentage was increased by subjects increasing .in most cases of pregnant women that in third trimester the level of serum iron ,serum ferritin and TS% was low . The percentage of anemia and IDA was high in third trimester of pregnancy in those subject group despite their doses of prophylactic iron therapy and this may be due to that those women may be not taking the prophylactic iron dose regularly from the first trimester also increase the plasma volume and red cell mass , and increasing of gestational age , all contribute to increase the percentage(Bashiri A, *et al* 2003). The incidence of anemia and IDA was significantly higher in women in the third trimester of pregnancy compare to those in first and second trimester in pregnant without iron prophylactic subjects group .This finding is consistent with a retrospective cohort study in Saudi Arabia demonstrated that iron deficiency was significantly associated with those who are in the third trimester of and non-complaint to iron supplementation (WHO 2001).

As for spacing, the highest percentage was found among pregnant women who had spacing of less than one year and up to one year .The lowest prevalence was observed among those whose spacing was more than two years . The severity of anemia in pregnant women with iron therapy was decreased than other subject groups, and it was high in pregnant women without iron therapy. From previous result we noted that birth spacing and iron supplementation have positive correlation with serum iron, serum ferritin, and TS % .while gravidity have negative correlation with these parameters . The effect of iron supplementation as general significantly on serum iron, serum ferritin and TS% has been observed. But unfortunately most women started iron dose after fourteen weeks because their normal nausea due to pregnancy get worsen.

The characteristics iron deficiency anemia observed in this study did not differ from those of other studied elsewhere, the hematological indices and clinical manifestations of iron deficiency anemia in pregnant women in Asir region (Kingdom of Saudi Arabia) were detected by Ahmed Mahfouz, et al 1994, investigated Saudi pregnant women, Hemoglobin concentrations (Hb), red blood cell count (RBC) and packed cell volume (PCV) were significantly lower in the pregnant women(Ahmed Mahfouz *et al* .1994).

This study was compared with other studies done in India . Bentely, *et al*.(2002), found that most common type of anemia among women was iron deficiency anemia, prevalence of anemia was high among all women. In 32.4% of women had mild (10–10.9 g/dl for pregnant women, 10–11.9g/dl for non-pregnant women), 14.19% had moderate (7–9.9 g/dl), and 2.2% had severe anemia (<7 g/dl) (Bentely , *et al* 2002).

4.2 Conclusion

The study concluded that:

- (1) The mean age among non-pregnant women were 33.2 ± 4.3 years and the mean age among pregnant women were 29.6 ± 4.4 years, the majority of study groups in the age group of 26-35 years .
- (2) The most Common type of anemia was iron deficiency anemia.
- (3) The Percentage of iron deficiency anemia was less in pregnant women who take iron therapy .
- (4) The mean of erythrocyte count in both non pregnant women and pregnant women with iron treatment was in the normal range, and slightly decreased in pregnant women without treatment..
- (5) Dimorphic blood picture was found in the thin blood film of pregnant women with iron therapy.
- (6) The most cause of iron depletion in all type of group study was insufficient iron intake.
- (7) The percentage of iron deficiency anemia was increased during third trimester .

4.3 Recommendations

- It is very necessary to give iron supplement to women before pregnancy and during pregnancy up to delivery to improve their iron status and reduce the incidence of iron deficiency anemia.
- Laboratory measures of iron stores should be included in antenatal care protocol of pregnant women for early diagnosis and the monitoring of pregnant women with iron deficiency anemia and Screening for iron deficiency in high risk groups should be considered.
- Educating of women's during and before the antenatal period, family planning and birth spacing during antenatal visit is needed.
- Nutritional education programs should be conducted especially for the women of child bearing age to advocate healthy dietary habits.
- Fortification of some food such as salts, flour, and juices can help to reduce the common problem.
- Future research is needed to evaluate dietary iron adequacy in Saudi Arabia.

References

- Ahmed AR Mahfouz , Mourad M El-Said ,Wole Al-Ekaija , Ibrahim A Al-Badawi ,RAG Al-Erian, Mohammed Abulmoneimet . Anemia among pregnant women in the Asir region Saudi Arabia 1994. Primary health care department.
- Adamson, JW . The relationship of erythropoietin and iron metabolism to red blood cell production in humans 1994 . Semin Oncol 21(2 suppl 2): 9-15
- Aisen, P, Wessling-Resnick, M ,Leibold ,E, A . Iron metabolism. Current Opinion in Chemical Biology 1999 ;3: 200-206.
- Al-Shehri S. Health Profile of Saudi adolescent schoolgirls . Presidency of girl's education, health affairs directorate 1996.
- American society of hematology. (studies on iron absorption) Published on 1963.
- Bailey, S., Evans RW Garratt RC, Gorinsky B , Hasnain SS , Horsburgh C, Jhoti H, Lindly PF , Mydin A, Sarra R , Watson JL . Molecular structure of serum transferrin at 3.3-Å. Resolution Biochemistry 1988 ;27 5804-5812.
- Bashiri A, Burstein E Sheiner E, Mazor M Anemia during pregnancy and treatment with intravenous iron: review of the literature. Eur J Obstet Gynecol Reprod Biol. 2003;110(1):2–7
- Baldwin, J. Chothia , C. Haemoglobin: the structural changes related to ligand binding and its allosteric mechanism. Journal of Molecular Biology 1979; 129: 175-220.
- Baynes, R.D , Bothwell, T.H. . Iron Deficiency. Annual Review of Nutrition 1990; 10: 133-148
- Bleackley MR, Wong AY, Hudson DM, Wu CH, Macgillivray RT. Transfus Med Rev. 2009 Apr;23(2):103-23
- Beutler, E., Hoffbrand, A.V. , Cook, J.D. . Iron deficiency and overload. Hematology/the Education Program of the American Society of Hematology 2003. pp 40-61.

Breymann C, Bian X, Blanco-Capito LR. Expert recommendations for the diagnosis and treatment of iron-deficiency anemia during pregnancy and the postpartum period in the Asia-Pacific region. *Journal of Perinatal Medicine*. 2010;38:1-8

Bruno de Benoist, Erin McLean, Ines Egli , Mary Cogswell .worldwide prevalence of anemia 1993-2005 .Geneva world health organization 2008 .

Campbell, J.A . Prolonged alterations of oxygen pressure in the inspired air with special reference to tissue oxygen tension, tissue carbon dioxide tension and haemoglobin. *Journal of Physiology* 1927 62: 211-231.

Centres for Disease Control and Prevention. Recommendations to Prevent and Control Iron Deficiency in the United States. Morbidity and Mortality. Weekly Report. 1998;47(No. RR-3)

Corazza GR, Valentini RA, Andreani ML, D'Anchino M, Leva MT, Ginaldi L, De Feudis L, Quaglino D, Gasbarrini G. . Subclinical coeliac disease is a frequent cause of iron-deficiency anaemia. *Scand J Gastroenterol* 1995.

C. G. Douglas, J. S. Haldane, and J. B. S. Haldane. The laws of combination of haemoglobin with carbon monoxide and oxygen. *Journal of Physiology* 1912; 44: 275-304

Dunn LL, Suryo Rahmanto Y, Richardson DR. Iron uptake and metabolism in the new millennium. *Trends in Cell Biology* 2007 Feb 17: 93-100

Fandrey, J. Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 2004; 286: R977-988.

Faura, J., J. Ramos, C. Reynafarje, E. English, P. Finne and C.A. Finch. Effect of altitude on erythropoiesis 1969. *Blood*, 33: 668-676

Frank Firkin, C.Chesterman, D.Penington, B.Rush , De Gruchy *Clinical Haematology in Medical Practice*. Fifth edition. London. Blackwell Publishing Ltd 1990. p29-32,172- 181.

Frazer, D.M. , Anderson, G.J. Iron Imports. I. Intestinal iron absorption and its regulation. American Journal Physiology Gastrointestinal Liver Physiology 2005;289: G631- 635.

Fleming, R.E. & Bacon, B.R. Orchestration of Iron Homeostasis. The New England Journal of Medicine 2005; 352 1741-1744.

Forget, B.G. Molecular Genetics of Human Hemoglobin Synthesis. Annals of Internal Medicine 1979; 91: 605-616.

Ganz, T. Heparin, a key regulator of iron metabolism and mediator of anemia of inflammation 2003. Blood 102 783-788.

Goddard AF, McIntyre AS, Scott BB.et al. (2000) Guidelines for the management of iron deficiency anaemia. British Society of Gastroenterology.Gut.46 Suppl 3-4:IV1–IV5

Greer. John P, John Foerster, Lukens. et al. (2003). Wintrobe's Clinical Hematology. 11th edition. New York. Lippincott Williams & Wilkins Publishers.

Harvey, P.W.J., Dexter, P.B. , Darnton Hill, I. (2000). The impact of consuming iron from non-food sources on iron status in developing countries. Publications in Healthy Nutrition 2000; 3: 375–383

Hassani. Ghazawi, Emad E. Eid , Mohammed M. Hussein Anemia in pregnancy in Jeddah Saudi Arabia. An epidemiological study,bull high inst public health.1988,18:541-53

Hill, A.V. The Combinations of Haemoglobin with Oxygen and with Carbon Monoxide. I. The Biochemical Journal 1913 ;7: 471-480.

Hoffbrand A.V, P.A. H. Moss, J.E. Pettit. Essential Haematology Fifth edition2006. Victoria, Australia. Blackwell Publishing Ltd, p24-26..

INACG, WHO, UNICEF . Guidelines for use of iron supplements to prevent and treat iron deficiency anemia. Report of a joint INACG/WHO/UNICEF/consultation. Geneva: World Health Organization 1998.

Instructor's Manual and Test Item File for Sylvia S. Mader Inquiry Into Life eighth Edition, 381 pages, Wm. C. Brown Publishers, Dubuque, IA (non-major's text) 1996.

Johanne Christiansen, C. G. Douglas , J. S. Haldane. The absorption and dissociation of carbon dioxide by human blood. Journal of Physiology 1914; 48: 244-271.

Johnson TA. Anaemia. In: Luesley DM, Baker PN, editors. Obstetrics and Gynaecology An evidencebased text for MRCOG. 2nd ed. London: Hodder Arnold; 2010; p. 139-43.

Kepczyk T, Kadakia SC . Prospective evaluation of gastrointestinal tract in patients with iron-deficiency anemia 1995.Dig Dis sci.40:1283–1289.

Latunde-Dada, G.O., Van der Westhuizen. Molecular and Functional Roles of Duodenal Cytochrome B (Dcytb) in Iron Metabolism. Blood Cells, Molecules, and Diseases 2002; 29: 356-360.

Leaper M, Johnston MJ, Barclay M, Dobbs BR, Frizelle FA . Reasons for failure to diagnose colorectal carcinoma at colonoscopy.Endoscopy.2004;36:499–503.

London IM, Bruns GP, Karibian D. The Regulation of Hemoglobin Synthesis and the Pathogenesis of Some Hypochromic Anemias. Medicine 1964;43: 789-802.

Lynch SR, Dassenko SA, Cook JD . M A Juillerat, R F Hurrell. Inhibitory effect of a soybean-protein - related moiety on iron absorption in humans. Am J Clin Nutr 1994; 60:567-572

Lynch, S.R. Interaction of iron with other nutrients. Nutrition Reviews April 1997; 55: 102-110.

Macdougall, L. G , Anderson , R. R , Mc Nab , G.M , Katz, J . The immune response in iron-deficient children: Impaired cellular defense mechanisms with altered humoral components. Journal of Pediatrics 1975; 86: 833-843.

M E Bentely , P L Griffiths . The burden of anemia among women in india . European Journal of Clinical Nutrition 2003 ; **57**, 52–60. doi:10.1038/sj.ejcn.1601504

McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A, Peters TJ, Raja KB, A.T. An Iron-Regulated Ferric Reductase Associated with the Absorption of Dietary Iron. Science 2001; 291: 1755-1759.

McLaren, G.D., Muir, W. A , Kellermeyer, R.W. Iron overload disorders: natural history, pathogenesis, diagnosis, and therapy. Critical Reviews in Clinical Laboratory Sciences 1983; 19: 205-266.

McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nature Medicine 1995; 1: 460-463.

McIntyre AS, Long RG . Prospective survey of investigations in outpatients referred with iron deficiency anaemia 1993 .Gut.34:1102–1107.

Milman N. Prepartum anaemia: prevention and treatment. Annals of Haematology. 2008;87:949-59.

Pak, M., Lopez, M. A., Gabayan, V., Ganz, T., Rivera, S. Suppression of hepcidin during anemia requires erythropoietic activity 2006 . Blood 108: 3730-3735.

Pavord S, Myers B, Robinson B, Allard S, Strong J, Oppenheimer C. UK guidelines on the management of iron deficiency in pregnancy. Br J Haematol. 2012;156(5):588-600.

Perutz, M.F. Review Lecture: Stereochemical Mechanism of Oxygen Transport by Haemoglobin", Proceedings of the Royal Society of London 1980 . Series B 208: 135-162.

Ronald Hoffman MD , Edward J. Benz Jr. MD , Leslie E. Silberstein MD , Helen Heslop MD , Jeffrey Weitz MD Hematology: Basic Principles and Practice 2008. Fifth edition. New York. Churchill Livingstone.

Roughton, F.J. Some recent work on the interactions of oxygen, carbon dioxide and haemoglobin. *The Biochemical Journal* 1970 ; 117: 801-812.

Reveiz L, Gyte GMI, Cuervo LG. Treatments for iron-deficiency anaemia in pregnancy. *The Cochrane Database of Systematic reviews*. 2007

Sarika Arora and Raj Kumar Kapoor . *Iron Metabolism in Humans: An Overview, Iron Metabolism* 2012.

Sayer JM, Long RG . A perspective on iron deficiency anaemia 1993 .*Gut*.34:1297–1299.

Shayeghi, M., Latunde-Dada, G.O., et al. (2005). Identification of an intestinal heme transporter. *Cel* 122: 789-801.

Schwartz, H.C., Goudsmit, R., Hill, R.L., Cartwright, G.E. , Wintrobe, M.M. The biosynthesis of hemoglobin from iron, protoporphyrin and globin 1961 . *Journal of Clinical Investigation* 40: 188-195

Skikne BS . Serum transferrin receptor . *Am J Hematology* 2008 ; .83:872–875.

Thomas H. Bothwell, R. W. Charlton, J. D. Cook and C. A. Finch 1979, *iron metabolism in man* .oxfaod: Blackwell Scientific Publications .

Thomas H. Bothwell Overview and mechanisms of iron regulation . *Nutrition Reviews* 1995; 53: 237-245.

Verster A, Pols J . Anemia in the Mediterranean Region. *Eastern Mediterranean Health Journal* 1995 ;1: 64-79.

Viatte L, Lesbordes-Brion JC, Lou DQ, Bennoun M, Nicolas G, Kahn A, Canonne-Hergaux F, Vaulont S. Deregulation of proteins involved in iron metabolism in hepcidin-deficient mice
Blood. 2005 Jun 15;105(12):4861-4. Epub 2005 Feb 15

White, J.C. , Beaven, G.H. A Review of the Varieties of Human Haemoglobin in Health and Disease 1954 . Journal of Clinical Pathology 7: 175-200.

World Health Organization. Turning the tide of malnutrition: responding to the challenge of the 21st century. Geneva, Switzerland 2000.

WHO Report . The prevalence of anemia in women: a tabulation of available information. Geneva: World Health Organization 1992 ; (document WHO/MCH/MSM/92.2).

WHO, UNICEF . Guidelines for the control of iron deficiency in countries of the Eastern Mediterranean, Middle East, and North Africa 1995 . Report of a joint WHO/UNICEF consultation. Iran: WHO-EM/NUT/177,E/G/11.96.

Yates JM, Logan EC, Stewart RM . Iron deficiency anaemia in general practice 2004: clinical outcomes over three years and factors influencing diagnostic investigations. Postgraduate Med J..80:405–410.

Zoller H, Koch RO, Theurl I, Obrist P, Pietrangelo A, Montosi G, Haile DJ, Vogel W, Weiss G . Expression of the duodenal iron transporters divalentmetal transporter 1 and ferroportin 1 in iron deficiency and iron overload", Gastroenterology 2001 ;120: 1412-1419.

Appendix 1

SUDAN UNIVERSITY OF SCIENCES & TECHNOLOGY
Types of anemia in pregnant and non-pregnant women in El-khormah province western
Saudi Arabia.
QUESTIONNAIRE

Patient Name: _____ Age: _____: ID _____

Date of birth: _____ Nationality _____ Tribe _____

Address: _____

Past medical history: _____

Blood disorders: Yes _____ NO _____ Chronic disease Yes _____ No _____

Past history of any type of anemia: Yes _____ No _____

If yes specify: _____ Physical examination: _____

Headache? Yes _____ No _____ Lack of concentration? Yes _____ No _____

Pallor? Yes _____ No _____ Medication antacid? Yes _____ No _____

Medical prenatal care started: Second or third trimester or no appointment yet ?

History of preterm birth one or more ? _____

Interval between pregnancies? Less than 12 month or more? _____

Receiving special supplemental nutrition program ? _____

Eating red meat? No _____ Infrequently _____ Frequently _____

Eating vegetables? No _____ Infrequently _____ Frequently _____

Drinking coffee / cocoa directly after meal? No _____ Infrequently _____ Frequently _____

Drinking brown tea? No _____ within meal _____ after meal

Dieting? Yes _____ No _____

Is the patient transfusion dependent? Yes _____ No _____

Is the patient treated with iron injection or iron therapy? Yes _____ No _____

Is the patient craving or chewing ice? Yes _____ No _____

Appendix 2

The all results of the study population

1- Non pregnant women

Age	RBC	Hb	HCT	MCV	MCH	MCHC	RDW	Retics	S. Iron	TIBC	S. Ferritin	TS	Hb. Electrophoresis
33	4.29	13.2	38.1	88.9	30.1	33.9	13.1	1.1	95.4	280	37.4	34	N
35	4.7	13.6	42.6	90.6	28.6	31.6	12.7	1.2	93.6	281	41.2	33	N
29	4.1	13.1	38.8	91.2	28.4	22.3	14	0.9	85.7	270	36.4	32	N
28	4.4	11.5	33.4	72.2	26.9	30.2	14.2	1	82.9	400	9.7	21	N
41	4.14	12.2	34.3	82.8	30.4	32.1	12.2	1.1	48.4	395	28.4	12	N
39	4.56	11.6	33.4	73.2	26.4	30.8	14.8	1.2	75.5	283	9.8	27	N
34	4.12	12.5	36.6	88.9	27.9	31.4	14.4	0.8	95.4	391	15.7	24	N
33	4.15	12.2	37.4	87.5	31	35.5	14.5	1.1	93.6	277	20.1	34	N
28	4.53	14.1	42.3	93.4	31.1	33.3	15	1.2	85.7	283	25.4	30	N
25	3.97	12	37.5	94.4	30.2	32	11.8	1	82.9	287	22.7	29	N
32	3.65	12.7	35.3	96.6	31.8	32.9	13	1.1	96	280	15.7	34	N
21	3.72	10.8	29.7	75.8	25.5	32	13.2	1.2	95.4	279	8.7	34	N
33	3.78	12.1	35	92.6	31.2	33.7	14.3	0.9	93.6	278	14.1	34	N
28	4.08	12.4	38.1	93.5	30.4	32.5	13.8	1	85.7	279	15.1	31	N
26	4.08	12.8	39.6	97	31.4	32.3	13.3	1.1	82.9	280	17.1	30	N
38	4.05	11.6	33.6	83	29.2	32.4	14.1	1.2	95.4	420	10.5	23	N
26	4.6	10.7	29.8	73.4	24.1	32.9	14.3	0.8	93.6	387	9.9	24	N
31	3.84	10.2	29.3	70.3	26.6	24.5	14	1.1	85.7	385	9.2	22	N
23	3.94	12.2	35.4	84.3	30.1	35.5	14.1	1.2	82.9	281	19.6	29	N
32	4.74	13	39.4	83.1	29.8	33	13.9	1	76.8	278	18.7	28	N
31	3.84	13.1	38.6	92.4	29.1	34.1	13.9	1.1	86.7	279	19.4	31	N
25	4.28	11.4	32.6	76.2	27.4	33.4	14.5	1.2	85.6	387	10.2	22	N
19	3.3	11.2	32.1	82	27.1	33.3	13.2	0.9	82.4	388	10.4	21	N

30	4.21	10.7	31.2	54.8	26.3	28.1	14.8	1	95.4	401	8.7	24	N
45	4.11	12.3	36.5	88.9	29.9	33.7	13.4	1.1	93.6	281	16.4	33	N
29	4.82	10.9	34.2	70.9	25.4	28.1	14.2	1.2	85.7	395	7.6	22	N
25	4.03	13.7	38.9	86.9	29.8	34.3	14	0.8	82.9	277	17.8	30	N
28	4.4	12.3	35.6	84	29.8	31.2	14.1	1.1	77.4	279	10.1	28	N
31	4.1	11.7	35.2	84.2	28.1	30.1	14.8	1.2	85.6	389	9.9	22	N
24	4.73	12.8	38.5	84	33.2	31.7	14.7	1	82.4	276	17.5	30	N
29	3.7	12.2	35.5	96	30.8	32.1	12.5	1.1	95.4	278	14.5	34	N
30	4.07	12.4	36.8	90.4	31.7	31.3	14.2	1.2	93.6	279	10.7	33	N
22	4.82	11.8	34.9	83	28	29.5	15.1	0.9	85.7	386	13.2	22	N
31	4.08	12.1	36.9	90.5	31.1	32.2	13.8	1	82.9	280	14.5	30	N
25	3.57	10.8	29.8	77.5	26.5	31	14.9	1.1	77.9	390	16.5	20	N
33	4.61	11.4	34.8	75.5	27.4	29.9	14.8	1.2	76.8	385	18.9	20	N
31	4.27	12.1	38.1	89.3	28.3	31.8	13.3	0.8	69.8	277	22.1	25	N
41	4.39	11.6	36.1	86.5	28.1	30.5	14.2	1.1	46.4	395	19.8	12	N
31	4.07	10.3	29.7	73	26.1	30.6	15.1	1.2	95.4	395	16.7	24	N
34	4.43	13.2	38	85.7	30.3	32.6	14.1	1	93.6	276	14.7	34	N
33	3.91	11.8	33.6	83	29.7	32.4	13.8	1.1	85.7	275	11.7	31	N
22	4.29	13.5	38.1	88.9	31.2	33.9	14.3	1.2	82.9	280	37.4	30	N
31	4.7	13.6	43.1	90.6	34.1	31.6	14.1	0.9	77.4	291	41.2	27	N
33	4.1	13.2	37.8	75.4	31.2	22.3	14.2	1	85.6	282	36.4	30	N
30	4.4	11.4	33.4	72.2	26.1	30.2	14.3	1.1	82.4	390	10.7	21	N
31	4.14	11	34.3	82.8	26.6	32.1	12.2	1.2	95.4	395	13.2	24	N
32	4.56	10.3	31.2	73.2	22.6	30.8	14.4	0.8	93.6	387	14.5	24	N
35	4.12	12.4	36.6	88.9	27.9	31.4	14.4	1.1	85.7	278	16.5	31	N
32	4.15	12.2	37.4	87.5	31	35.5	14.5	1.2	82.9	277	18.9	30	N
33	4.53	14.1	42.3	93.4	31.1	33.3	14.1	1	77.4	283	22.1	28	N
37	3.97	12.8	37.5	94.4	30.2	32	11.8	1.1	80.2	285	19.8	28	N

41	3.65	12.8	35.3	96.6	31.8	32.9	13	1.2	65.4	280	16.7	23	N
35	3.72	10.2	29.7	75.8	25.5	32	14.5	0.9	49.7	389	14.7	13	N
32	3.78	11.8	35	79.8	26.8	33.7	14.3	1	95.4	399	11.7	24	N
36	4.08	14.2	43.2	93.5	30.4	32.5	13.8	1.1	93.6	279	37.4	33	N
31	4.08	12.8	39.6	97	31.4	32.3	13.3	1.2	85.7	280	41.2	30	N
28	4.05	10.9	33.6	82.1	26.9	32.4	14.1	0.8	82.9	401	36.4	21	N
30	4.6	9.8	29.8	73.4	24.1	32.9	14.7	1.1	77.4	395	10.7	20	N
33	3.84	9.4	27	70.3	26.5	24.5	14.5	1.2	85.6	390	13.2	22	N
31	3.94	12.8	37.2	84.3	29.9	35.5	14.1	1	82.4	279	14.5	29	N
36	4.74	13	39.4	83.1	27.4	33	14.4	1.1	95.4	278	16.5	34	N
35	3.84	13.6	40.1	92.4	31.5	34.1	14.1	1.2	93.6	277	18.9	34	N
37	4.28	10.9	32.6	76.2	25.5	33.4	14.3	0.9	85.7	394	22.1	22	N
33	3.3	10.7	32.1	82	26.8	33.3	13.2	1	82.9	392	19.8	21	N
29	4.21	6.5	20.1	54.8	15.4	28.1	15.1	1.1	82.4	401	16.7	21	N
31	4.11	12.3	36.5	88.9	29.9	33.7	13.4	1.2	77.9	281	14.7	28	N
33	4.82	9.6	30.3	70.9	19.9	28.1	14.2	0.8	76.8	280	11.7	27	N
31	4.03	12	35	86.9	29.8	34.3	14.6	1.1	59.6	278	37.4	21	N
27	4.4	12.6	35.6	84	28.1	31.2	14.1	1.2	68.7	277	41.2	25	N
33	4.1	10.6	33.1	82.1	25.4	30.1	14.3	1	38.6	396	36.4	10	N
31	4.73	12.2	38.5	81.3	27.2	31.7	14.7	1.1	77.4	276	17.5	28	N
31	3.7	11.4	35.5	82.2	26.9	32.1	12.5	1.2	49.5	278	10.7	18	N
30	4.07	12.7	36.8	90.4	28.3	31.3	14.2	0.9	95.4	279	13.2	34	N
32	4.82	10.3	31.2	72.5	21.4	29.5	14.5	1	93.6	386	14.5	24	N
33	4.08	11.9	36.9	90.5	29.2	32.2	13.8	1.1	85.7	280	16.5	31	N
31	3.57	8.6	26.4	77.5	24.1	31	14.3	1.2	82.9	390	18.9	21	N
32	4.61	10.4	33	75.5	22.6	29.9	14.3	0.8	77.4	385	22.1	20	N
30	4.27	13.4	38.1	89.3	28.3	31.8	13.3	1.1	85.6	277	19.8	31	N
21	4.39	11.6	36.4	82.8	26.4	30.5	13.1	1.2	82.4	378	16.7	22	N

31	4.07	9.1	28.1	73	22.4	30.6	12.7	1	95.4	395	14.7	24	N
31	4.43	13.8	40.2	85.7	28	32.6	14	1.1	93.6	279	11.7	32	N
35	3.91	10.9	33.6	82	26.1	32.4	14.2	1.2	85.7	396	37.4	22	N
32	4.29	12.9	38.1	88.9	30.1	33.9	12.2	0.9	82.9	280	41.2	30	N
36	4.7	13.6	43.1	90.6	28.6	31.6	14.8	1	74	291	36.4	25	N
34	4.1	13.1	40	75.4	31.1	22.3	14.4	1.1	80.2	282	36.4	29	N
36	4.4	10.1	31.5	72.2	21.9	30.2	14.5	1.2	85.6	380	9.7	22	N
30	4.14	11	34.3	82.8	27.1	32.1	15	0.8	82.4	385	28.4	21	N
34	4.56	10.3	32.8	73.2	22.6	30.8	11.8	1.1	77.9	390	10.7	20	N
31	4.12	12.3	36.6	88.9	27.9	31.4	13	1.2	76.8	278	13.2	28	N
32	4.15	13.4	39.7	87.5	31	35.5	13.2	1	81	277	14.5	29	N
33	4.53	14.1	42.3	93.4	31.1	33.3	14.3	1.1	82.4	283	16.5	29	N
31	3.97	12	37.5	94.4	30.2	32	13.8	1.2	54	276	18.9	20	N
34	3.65	11.6	35.3	82.4	26.5	32.9	13.3	0.9	85.6	370	22.1	23	N
33	3.72	9.5	29.7	75.8	25.5	32	14.1	1	82.4	389	19.8	21	N
35	3.78	11.8	35	92.6	31.2	33.7	14.3	1.1	77.9	278	16.7	28	N
26	4.08	12.8	38.1	93.5	30.4	32.5	14	1.2	76.8	279	14.7	28	N
37	4.08	12.5	39.6	97	31.4	32.3	14.1	0.8	62.4	280	11.7	22	N
29	4.05	10.9	33.6	82.8	26.9	32.4	13.9	1.1	38.9	277	37.4	14	N
31	4.6	9.8	29.8	73.4	24.1	32.9	13.9	1.2	95.4	388	41.2	24	N
42	3.84	9.4	27	70.3	27	24.5	14.5	1	93.6	390	36.4	24	N
33	3.94	12.4	35.9	84.3	29.9	35.5	13.2	1	85.7	279	19.6	31	N
35	4.74	13	39.4	83.1	30.1	33	14.8	1.1	82.9	278	18.7	30	N
39	3.84	12.1	35.5	92.4	31.5	34.1	13.4	1.2	77.4	277	19.4	28	N
28	4.28	10.9	32.6	76.2	25.5	33.4	14.2	0.9	85.6	387	10.2	22	N
32	3.3	10.7	32.1	82.4	25.1	33.3	14	1	82.4	390	10.4	21	N
19	4.21	10.2	32.1	54.8	17.2	28.1	14.1	1.1	95.4	401	10.7	22	N
31	4.11	12.3	36.5	88.9	30.1	33.7	14.8	1.2	93.6	281	13.2	33	N

34	4.82	9.6	30.5	70.9	25.6	28.1	14.7	0.8	85.7	395	14.5	22	N
30	4.03	13.9	41	86.9	31	34.3	12.5	1.1	82.9	277	16.5	30	N
32	4.4	11.1	34.4	70.5	26.8	31.2	14.2	1.2	82.4	385	18.9	21	N
30	4.1	10.6	33.1	84.2	28.1	30.1	15.1	1	54	389	22.1	14	N
35	4.73	14.1	38.5	81.3	31	31.7	13.8	1.1	85.6	276	19.8	31	N
32	3.7	12.4	37	96	27.2	32.1	14.9	1.2	82.4	278	16.7	30	N
41	4.07	12.9	38.2	90.4	30.8	31.3	14.8	0.9	77.9	279	14.7	28	N
35	4.82	10.3	32.1	72.5	26.2	29.5	13.3	1	76.8	386	11.7	20	N
39	4.08	11.9	36.9	90.5	27.2	32.2	14.2	1.1	62.4	382	37.4	16	N
38	3.57	8.6	26.3	77.5	29.2	31	15.1	1.2	38.9	390	41.2	10	N
37	4.61	10.4	32.1	75.5	24.1	29.9	14.1	0.8	85.6	385	36.4	22	N
36	4.27	14.9	43.2	89.3	31.1	31.8	13.8	1.1	82.4	277	16.5	30	N
35	4.39	11.6	35.6	86.5	28.3	30.5	14.3	1.2	77.9	378	11.2	21	N
34	4.07	10.2	29.7	73	26.4	30.6	14.1	1	76.8	395	10.7	20	N
39	4.43	12.4	38	85.7	27.6	32.6	14.2	1.1	55.1	276	13.2	20	N
38	3.91	11.8	33.6	85.9	28	32.4	14.3	1.2	39.4	275	14.5	14	N
37	4.29	14.7	42.5	88.9	27.9	33.9	12.2	0.9	44.7	383	16.5	12	N
34	4.7	13.6	43.1	90.6	30.1	31.6	14.4	1	95.4	278	18.9	34	N
35	4.1	13.9	42.9	75.4	28.6	22.3	14.4	1.1	93.6	280	22.1	33	N
39	4.4	10.1	31.4	72.2	25.1	30.2	14.5	1.2	85.7	389	19.8	22	N
38	4.14	11	34.3	82.8	26.8	32.1	14.1	0.8	82.9	283	16.7	29	N
37	4.56	10.3	32	73.2	27.1	30.8	11.8	1.1	77.4	385	14.7	20	N
34	4.12	11.5	36.6	88.9	27	31.4	13	1.2	85.6	275	11.7	31	N
39	4.15	13.2	37.4	87.5	27.9	35.5	14.5	1	82.4	277	37.4	30	N
38	4.53	14.1	42.3	93.4	31	33.3	14.3	1.1	95.4	283	41.2	34	N
35	3.97	12	37.5	94.4	31.1	32	13.8	1.2	93.6	276	36.4	34	N
36	3.65	11.6	35.3	82	26.1	32.9	13.3	0.9	85.7	370	15.7	23	N
34	3.72	12.1	35.6	75.8	31.8	32	14.1	1	82.9	389	12.4	21	N

41	3.78	11.8	35	92.6	27	33.7	14.7	1.1	82.4	278	14.1	30	N
42	4.08	13.1	38.1	93.5	31.2	32.5	14.5	1.2	77.9	279	15.1	28	N
40	4.08	12.8	39.6	97	30.4	32.3	14.1	0.8	76.8	280	17.1	27	N
36	4.05	10.9	33.6	28.1	26.8	32.4	14.4	1.1	82.4	395	13.2	21	N
35	4.6	11.2	33.2	73.4	26.9	32.9	14.1	1.2	54	360	10.7	15	N
39	3.84	10.8	31	70.3	24.1	24.5	14.3	1	85.6	388	13.2	22	N
36	3.94	12.3	37	84.3	27	35.5	13.2	1	82.4	279	14.5	30	N
38	4.74	13	39.4	83.1	29.9	33	15.1	1.1	77.9	278	16.5	28	N
29	3.84	12.9	36.4	92.4	30.1	34.1	13.4	1.2	76.8	275	18.9	28	N
37	4.28	11.9	34.1	76.2	31.5	33.4	14.2	0.9	62.4	350	22.1	18	N
25	3.3	10.7	32.1	82	25.5	33.3	14.6	1	38.9	379	19.8	10	N
38	3.65	7.2	22	54.8	25.1	28.1	14.1	1.1	85.6	401	16.7	21	N
37	4.11	12.7	36.5	88.9	28.2	33.7	14.3	1.2	82.4	281	14.7	29	N
34	4.82	10.4	31.5	70.9	19.9	28.1	14.7	0.8	77.9	395	11.7	20	N
24	4.03	12.1	35	86.9	29.8	34.3	12.5	1.1	76.8	277	37.4	28	N
35	4.4	11.1	34.6	70.5	22	31.2	14.2	1.2	82.4	385	41.2	21	N
34	4.1	11.7	35.2	84.2	27	30.1	14.5	1	54	389	36.4	14	N
41	4.73	13.4	38.5	81.3	28.6	31.7	13.8	1.1	85.6	276	17.5	31	N
40	3.7	12.1	35.5	96	30.8	32.1	14.3	1.2	82.4	278	12.5	30	N
42	4.07	11.5	35.4	90.4	28.3	31.3	14.3	0.9	77.9	279	13.2	28	N
36	4.82	10.3	31.8	72.5	21.4	29.5	13.3	1	76.8	386	14.5	20	N
39	4.08	11.9	36.9	90.5	29.2	32.2	14.1	1.1	62.4	280	14.5	22	N
37	3.57	8.6	26.1	77.5	24.1	31	13.8	1.2	38.9	390	10.7	10	N
38	4.61	10.4	31.5	75.5	22.6	29.9	14.3	0.8	85.6	385	13.2	22	N
34	4.27	14.2	40.3	89.3	28.3	31.8	14.1	1.1	82.4	277	14.5	30	N
36	4.39	11.6	35.4	86.5	27	30.5	14.2	1.2	77.9	387	16.5	20	N
35	4.07	10.3	29.7	73	22.4	30.6	14.3	1	76.8	386	18.9	20	N
38	4.43	12.4	38	85.7	28	32.6	12.2	1.1	64	276	22.1	23	N

34	3.91	11.2	33.6	79.8	26.4	32.4	14.4	1.2	39.4	275	19.8	14	N
37	4.29	14.7	43.2	88.9	30.1	33.9	14.4	0.9	75.1	276	16.7	27	N
43	4.7	13.6	43.1	90.6	28.6	31.6	14.5	1	88.3	291	14.7	30	N
28	4.1	12.4	37.8	83	28	22.3	14.1	1.1	94.7	282	11.7	34	N
30	4.4	10.1	31.6	72.2	21.9	30.2	11.8	1.2	82.4	380	37.4	22	N
41	4.14	11.5	34.3	82.8	27	32.1	13	0.8	54	283	41.2	19	N
39	4.56	10.3	31.2	73.2	22.6	30.8	14.5	1.1	85.6	358	36.4	24	N
42	4.12	12.2	36.6	88.9	27.9	31.4	14.3	1.2	82.4	278	15.7	30	N
33	4.15	13.2	37.4	87.5	31	35.5	13.8	1	77.9	277	20.1	28	N
45	4.53	14.1	42.3	93.4	31.1	33.3	13.3	1.1	76.8	283	25.4	27	N
37	3.97	12	37.5	94.4	30.2	32	14.1	1.2	62.4	276	22.7	23	N
38	3.65	13.2	38.9	96.6	31.8	32.9	14.7	0.9	38.9	370	15.7	10	N
35	3.72	9.5	29.7	75.8	25.5	32	14.5	1	85.6	389	8.7	22	N
37	3.78	11.8	35	92.6	31.2	33.7	14.1	1.1	82.4	278	14.1	30	N
34	4.08	12.9	38.1	93.5	30.4	32.5	14.4	1.2	77.9	279	15.1	28	N
31	4.08	12.8	39.6	97	31.4	32.3	14.1	0.8	76.8	280	17.1	27	N
36	4.05	10.9	33.6	82	26.9	32.4	14.1	1.1	82.4	277	10.5	30	N
35	4.6	11.2	34.5	73.4	24.1	32.9	13.8	1.2	95.4	360	10.7	26	N
37	3.84	11.9	36.4	84	28	24.5	14.3	1	93.6	390	13.2	24	N
38	3.94	12.2	37.2	84.3	29.9	35.5	14.1	1	85.7	279	14.5	31	N
33	4.74	13.1	39.4	83.1	27.4	33	14.2	1.1	82.9	278	16.5	30	N
31	3.84	12.1	35.5	92.4	31.5	34.1	14.3	1.2	77.4	277	18.9	28	N
35	4.28	11.8	32.6	84	28	33.4	12.2	0.9	85.6	350	22.1	24	N
33	3.3	11.5	32.1	82.4	26.5	33.3	14.4	1	82.4	379	19.8	22	N
34	4.21	10.7	32.3	54.8	15.4	28.1	14.4	1.1	95.4	401	16.7	24	N
28	4.11	8.5	25.4	75.6	19.8	33.7	14.5	1.2	93.6	281	14.7	33	N
30	4.82	12.7	36.5	70.9	29.9	28.1	14.1	0.8	85.7	395	12.4	22	N
41	4.03	10.4	32.2	81.5	26.3	34.3	11.8	1.1	82.9	277	37.4	30	N

36	4.4	12.1	35.6	70.5	29	31.2	13	1.2	55	385	41.2	14	N
35	4.1	11.1	35.2	82.8	26.8	30.1	14.5	1	36.5	389	36.4	9	N
34	4.73	11.7	36.4	82.2	26.4	31.7	14.3	1.1	48.7	276	17.5	18	N
33	3.7	13.4	38.1	96	30.8	32.1	13.8	1.2	67.8	278	13.2	24	N
32	4.07	12.1	36.8	90.4	28.3	31.3	13.3	0.9	49.8	279	14	18	N
31	4.82	12.2	34.9	85	28	29.5	14.1	1	95.4	386	10.7	25	N
31	4.08	10.3	31.5	78.2	26.8	32.2	14.7	1.1	93.6	280	13.2	33	N
32	3.57	11.9	36.1	83.4	28	31	14.5	1.2	85.7	390	14.5	22	N
32	4.61	8.6	27.2	75.5	22.6	29.9	14.1	0.8	82.9	385	16.5	21	N
35	4.27	10.4	30.5	80.2	25.9	31.8	14.4	1.1	77.4	277	18.9	28	N
30	4.39	14.2	43.2	86.5	29.5	30.5	14.1	1.2	85.6	378	22.1	23	N
31	4.07	11.6	35.1	83	27.3	30.6	14.1	1	82.4	395	19.8	21	N
35	4.43	10.3	30.8	82.1	26.5	32.6	13.8	1.1	95.4	276	16.7	34	N
36	3.91	12.4	33.6	85.9	27.9	32.4	14.3	1.2	93.6	275	14.7	34	N
32	4.29	12	38.1	88.9	30.1	33.9	14.1	0.9	85.7	280	11.7	31	N
30	4.7	14.7	43.1	90.6	28.6	31.6	14.2	1	82.9	291	37.4	28	N
31	4.1	13.6	37.8	85.1	29.1	22.3	14.3	1.1	95.4	282	41.2	34	N
31	4.4	12.4	37.1	84.4	30.1	30.2	12.2	1.2	93.6	380	36.4	25	N
29	4.14	10.1	30.8	82.8	26.6	32.1	14.4	0.8	85.7	283	28.4	30	N
30	4.56	12.2	35.6	83.2	28.1	30.8	14.4	1.1	82.9	358	9.8	23	N
29	4.12	10.3	31.1	81.5	26.3	31.4	14.5	1.2	77.4	278	15.7	28	N
33	4.15	12.2	37.4	87.5	31	35.5	14.1	1	85.6	277	20.1	31	N
30	4.53	13.2	40.1	93.4	31.1	33.3	11.8	1.1	82.4	283	25.4	29	N
34	3.97	14.1	43	94.4	30.2	32	13	1.2	95.4	276	22.7	34	N
32	3.65	12	35.3	96.6	31.8	32.9	14.5	0.9	93.6	370	15.7	25	N
31	3.72	13.2	40.1	83.1	29.4	32	14.3	1	85.7	389	8.7	22	N
33	3.78	9.5	35	72.1	20.2	33.7	13.8	1.1	82.9	278	14.1	30	N
35	4.08	12.1	38.1	93.5	30.4	32.5	13.3	1.2	42.5	279	15.1	15	N

32	4.08	12.9	39.6	97	31.4	32.3	14.1	0.8	59.7	280	17.1	21	N
33	4.05	12.8	37.2	83	28	32.4	14.7	1.1	69.9	277	11.8	25	N
31	4.6	10.9	31.1	73.4	24.1	32.9	14.5	1.2	39.8	360	13.1	11	N
35	3.84	12	37.5	70.3	27	24.5	14.1	1	41.2	387	12.1	11	N
32	3.94	12.1	35.8	84.3	29.9	35.5	14.4	1.1	95.4	279	19.6	34	N
31	4.74	12.2	38.2	83.1	27.4	33	14.1	1.2	93.6	278	18.7	34	N
35	3.84	13.1	35.5	92.4	31.5	34.1	14.1	0.9	85.7	277	19.4	31	N
34	4.28	12.1	37.5	84.1	27.8	33.4	13.8	1	82.9	350	10.7	24	N
31	3.3	11.8	35.1	97.2	32.4	33.3	14.3	1.1	77.4	379	13.2	20	N
34	4.21	11.5	35.2	83	27	28.1	14.1	1.2	85.6	386	14.5	22	N
32	4.11	12.3	36.5	88.9	29.9	33.7	14.2	0.8	82.4	281	16.5	29	N
31	4.82	9.6	30.1	70.9	19.9	28.1	14.3	1.1	95.4	385	18.9	25	N
30	4.03	12	35	86.9	29.8	34.3	12.2	1.2	93.6	277	22.1	34	N
35	4.4	11.1	33.9	78.9	22	31.2	14.4	1	85.7	385	19.8	22	N
33	4.1	10.6	31.2	76.2	25.4	30.1	14.4	1.1	82.9	389	16.7	21	N
35	4.73	12.2	38.5	84	28.1	31.7	14.5	1.2	95.4	276	14.7	34	N
34	3.7	12	35.5	96	30.8	32.1	14.1	0.9	93.6	278	11.7	34	N
32	4.07	12	36.8	90.4	28.3	31.3	11.8	1	85.7	279	37.4	31	N
31	4.82	10.3	32.1	72.5	21.4	29.5	13	1.1	82.9	386	41.2	21	N
36	4.08	12.1	36.9	90.5	29.2	32.2	14.5	1.2	77.4	280	36.4	28	N
35	3.57	8.6	25.6	77.5	24.1	31	14.3	0.8	85.6	386	14.5	22	N
31	4.61	10.4	31.2	75.5	22.6	29.9	13.8	1.1	82.4	385	12.2	21	N
33	4.27	12.1	38.1	89.3	28.3	31.8	13.3	1.2	99.4	277	16.5	36	N
34	4.39	12	38	86.5	28	30.5	14.1	1	93.6	378	15.3	25	N
34	4.07	9.1	28.5	73	22.4	30.6	14.7	1.1	96.7	385	12.7	25	N
37	4.43	12.4	38	85.7	28	32.6	14.5	1.3	91.8	276	14.7	33	N
31	3.91	10.9	33.6	82	26	32.4	14.1	1	68.4	275	15.5	25	N
34	4.29	12.9	38.1	88.9	30.1	33.9	14.4	1.1	75.4	276	37.4	27	N

33	4.7	13.6	43.1	90.6	28.6	31.6	14.1	1.3	94	275	41.2	34	N
30	4.1	12.4	37.8	85.1	29.2	22.3	14.8	1.2	88	274	36.4	32	N
31	4.4	10.1	31.2	78.1	26.4	30.2	14.9	1.4	35.1	387	9.7	9	N

2- Pregnant women with iron therapy

Age	RBC	Hb g/dl	HCT%	MCV	MCH	MCHC	RDW	Retics	S.Iron	TIBC	S. Ferritin	TS	Hb electrophoresis
28	3.98	8.6	27.2	72.1	21.6	31.6	17.1	2.3	82.4	389	10.1	21	N
31	4.55	10.4	31.2	79.8	25.9	32.1	14.5	2.4	77.9	370	11.4	21	N
35	4.01	10.2	32.1	80.4	28.7	21.1	14.3	2.6	76.8	365	17.1	21	N
29	4.17	12.1	35.3	87.2	27.3	32.3	15.2	1.9	62.4	381	15.8	16	N
32	3.74	10.1	31	82.5	26.4	33.2	15.5	2.3	38.9	380	15.4	10	N
33	4.3	12.3	37.1	85.6	27.2	31.9	14.8	2.9	85.6	325	18.4	26	N
31	4.2	10.7	33.1	82	26.5	32.1	14	2.7	82.4	370	19.6	22	N
29	3.78	10.1	31	81	21.9	30.2	19.7	3.1	77.9	365	11.2	21	N
28	4.1	10.5	33.2	80.5	24.7	30.7	17.5	2.1	76.8	381	13.8	20	N
31	4.5	12.4	37.1	94	28	31.6	14.4	1.9	82.4	380	14.5	22	N
30	4.7	10.4	31.2	77	23.8	30.9	17.7	2.2	54	325	11.4	17	N
32	5.12	10.2	31.2	80.9	26.8	31.9	14.8	3.1	85.6	370	11.6	23	N
33	4.25	12.1	35.4	91.4	28.1	31.2	18.2	2.7	82.4	365	11.4	23	N
35	4.07	10.1	30.8	83.1	27	31.8	14.1	2.4	77.9	381	12.7	20	N
38	4.9	12.1	36.7	83.4	27.1	30.5	14.9	3.2	76.8	380	10.9	20	N
39	4.6	10.7	31.5	80.1	25.1	31.3	16.6	2.5	62.4	325	17.1	19	N
37	4.32	12.1	38.2	84.2	28.1	31.2	14.2	2.2	38.9	370	15.8	11	N
28	4.78	10.2	32.1	79.8	24.1	31.5	16.4	2.6	85.6	365	10.5	23	N
29	3.61	12.3	36.5	91.2	28	32.1	16.1	2.7	82.4	381	18.4	22	N
24	4.05	12.4	39.1	94.1	31.9	33	13.1	2	77.9	380	19.6	20	N

21	4.16	12.2	37.9	92.1	30.8	31.6	12.7	2.9	76.8	325	12.9	24	N
25	4.1	8.6	27.4	77.2	21.6	32.1	13.5	3.1	64	370	13.8	17	N
28	3.83	10.4	30.9	80.1	25.9	21.1	18.2	2.8	39.4	365	14.5	11	N
26	3.71	10.2	31.1	76.4	28.7	32.3	13.8	3.4	75.1	381	12.5	20	N
23	4.27	12.3	37.1	91.2	27.3	33.2	16.2	2.8	88.3	380	13.1	23	N
31	4.11	10.1	31	78	26.4	31.9	13.8	2.4	94.7	325	11.4	29	N
30	4.09	12.2	37.6	90.7	27	32.1	14.5	2.3	82.4	385	12.7	21	N
33	4.09	10.7	32.2	82.4	26.5	30.2	14.3	2.4	54	389	12.6	14	N
35	3.98	10.1	32	81.1	21.9	30.7	17.1	2.2	85.6	389	17.1	22	N
36	4.55	10.5	32.3	82.6	24.7	31.6	14.5	3.2	82.4	390	15.8	21	N
34	4.01	11.2	34.2	82.1	26.9	30.9	14.3	3.4	77.9	380	15.4	20	N
28	4.17	10.4	33.1	80.1	23.8	31.9	15.2	22	76.8	325	18.4	24	N
29	3.74	10.2	32.3	78.4	26.8	31.2	15.5	2.4	62.4	370	19.6	17	N
27	4.3	11.3	33.9	28.9	27	31.8	14.8	2.6	38.9	365	11.4	11	N
36	4.2	10.1	31.2	80.4	27	30.5	14	2.8	85.6	381	13.8	22	N
33	3.78	11.2	33.4	82.1	22.7	31.3	19.7	2.7	82.4	380	14.5	22	N
28	4.1	10.7	33.2	82.5	25.1	30.7	17.5	2.9	77.9	325	11.3	24	N
32	4.5	11.5	36.2	82.1	27.5	31.6	14.4	2.6	76.8	370	13.1	21	N
27	4.7	10.2	30.8	81.2	24.1	30.9	17.7	2.4	82.4	365	11.4	22	N
31	5.12	12.1	37.1	94.2	28	31.9	14.8	2.4	54	381	12.7	14	N
29	4.25	11.3	34	80.5	24.9	31.2	18.2	2.6	85.6	380	11.4	22	N
24	4.07	12.6	37.2	92.4	27	31.8	14.1	2.7	82.4	325	12.5	25	N
32	4.9	8.6	25.6	77	22.7	30.5	14.9	3.1	77.9	385	11.2	20	N
34	4.6	10.4	31.7	80.9	25.1	31.3	16.6	2.6	76.8	389	17.1	20	N
31	4.32	10.2	31.3	80	27.5	31.2	14.2	2.1	62.4	389	15.8	16	N
30	4.78	12.5	36.5	91.2	28.1	31.5	16.4	3.1	38.9	390	11.8	10	N
28	3.61	10.1	31.5	79.3	28	32.1	16.1	3.2	85.6	380	18.4	22	N
29	4.05	12.2	37.2	92.1	31.9	31.6	13.1	3.6	82.4	325	19.6	25	N

26	4.16	10.7	33.2	78.4	30.8	32.1	12.7	2.4	77.9	370	12.9	21	N
32	4.1	10.1	31.4	80.1	31.4	21.1	13.5	2.8	76.8	365	11.1	21	N
31	3.83	10.5	30.9	80.4	26.9	32.3	18.2	2.7	55	381	14.5	14	N
30	3.71	12	33.8	92.4	31	33.2	13.8	2.1	36.5	380	10.8	10	N
25	4.27	10.4	31	82.5	25.8	31.9	16.2	2.4	48.7	325	13.1	15	N
28	4.11	10.2	31.3	80.1	29.4	32.1	13.8	3.5	67.8	370	11.4	18	N
29	4.09	13	37.6	98.4	30.8	30.2	14.5	3.4	49.8	365	12.7	14	N
24	4.09	10.1	31.5	72.2	29.8	30.7	14.3	3.2	49	380	17.1	13	N
31	3.98	12	37.2	91.2	28	31.6	17.1	3.6	82.4	325	15.8	25	N
30	4.55	10.7	30.6	82.4	25.9	30.9	14.5	2.9	54	370	15.4	14	N
30	4.01	12.9	37	97.8	28.7	31.9	14.3	2.7	85.6	365	18.4	23	N
28	4.17	10.2	31	80.9	27.3	31.2	15.2	2.8	82.4	381	14.9	22	N
29	3.74	12.8	37.5	97	30.7	31.8	15.5	3.1	77.9	380	11.7	20	N
28	4.3	12.2	35.2	96.4	27.8	30.5	14.8	3.4	76.8	325	12.6	24	N
30	4.2	12.1	35.2	93.4	27.4	31.3	14	2.9	62.4	370	17.1	17	N
24	3.78	8.6	27.5	80.1	21.9	30.2	19.7	2.3	38.9	365	15.8	11	N
22	4.1	10.4	33.2	80.5	24.7	30.7	17.5	3.9	85.6	381	15.4	22	N
18	4.5	10.2	39.9	81.2	28	31.6	14.4	2.4	82.4	380	18.4	22	N
20	4.7	12.3	36.6	94	23.8	30.9	17.7	2.4	77.9	325	19.6	24	N
26	5.12	10.1	31	80.4	26.8	31.9	14.8	2.3	76.8	385	10.2	20	N
32	4.25	12.4	35.6	95.2	27.6	31.2	18.2	2.5	39.1	389	10.9	10	N
21	4.07	10.7	31.2	82.5	27	31.8	14.1	3.1	44	389	14.5	11	N
30	4.9	10.1	30.2	80.1	22.7	30.5	14.9	2.4	41	390	12.5	10	N
28	4.6	10.5	31	81	25.1	31.3	16.6	2.1	52.1	381	13.1	14	N
27	4.32	12.1	38.2	83.4	27.5	31.2	14.2	3.2	80.1	380	11.4	21	N
25	4.78	10.4	31.2	80.5	24.1	31.5	16.4	3.6	79.2	325	12.7	24	N
26	3.61	10.2	31.5	79.2	28	32.1	16.1	3.1	63.4	385	12.6	16	N
32	4.05	12.2	38.1	89.7	31.9	31.6	13.1	3	79	389	17.1	20	N

29	4.16	10.1	30.2	78.4	30.8	32.1	12.7	2.8	38.7	389	15.8	10	N
28	4.1	12.3	38.7	94.5	31.4	21.1	13.5	2.9	42.5	390	15.4	11	N
31	3.83	10.7	30.9	81.1	26.9	32.3	18.2	2.4	59.7	380	18.4	16	N
30	3.71	12.1	33.8	96.1	31	33.2	13.8	2.5	69.9	325	14.9	21	N
28	4.27	10.2	29.8	82.5	25.8	31.9	16.2	3.6	39.8	370	11.7	11	N
27	4.11	12.1	37	92.1	29.4	32.1	13.8	3.2	41.2	365	10.8	11	N
29	4.09	12.7	37.6	91.2	30.8	30.2	14.5	3.1	65.2	381	17.1	17	N
31	4.09	12.4	36.3	93.5	29.8	30.7	14.3	2.3	64	380	15.8	17	N
25	3.98	8.6	27.2	80.5	21.6	31.6	17.1	2.3	82	325	15.4	25	N
26	4.55	10.4	31.8	82.8	25.9	30.9	14.5	2.1	57.2	370	18.4	15	N
28	4.01	10.2	32.1	77	28.7	31.9	14.3	2	61	365	19.6	17	N
29	4.17	12.1	35.3	95.6	27.3	31.2	15.2	1.9	64.3	381	10.5	17	N
30	3.74	12.2	35.9	95.7	30.7	31.8	15.5	1.8	76	380	13.8	20	N
33	4.3	12.1	33.9	91.7	24.6	30.5	14.8	2.1	82.4	325	14.5	25	N
32	4.2	10.7	33.2	74.4	26.5	31.3	14	1.9	54	385	11.4	14	N
35	3.78	10.1	31.2	80.1	21.9	30.2	19.7	1.8	85.6	389	13.1	22	N
34	4.1	10.5	33.2	80.5	24.7	30.7	17.5	1.7	82.4	389	11.4	21	N
29	4.5	12.7	39.9	95.2	28	31.6	14.4	1.9	77.9	390	11.1	20	N
24	4.7	10.4	30.8	79.8	23.8	30.9	17.7	2.3	76.8	380	17.1	20	N
27	5.12	10.2	32.1	80.4	26.8	31.9	14.8	2.3	62.4	325	15.8	19	N
31	4.25	12.1	34	92.7	24.9	31.2	18.2	2.1	38.9	370	15.4	10	N
21	4.07	10.1	31.1	84.9	27	31.8	14.1	2	85.6	365	18.4	23	N
36	4.9	12	36.7	91.4	27.1	30.5	14.9	1.9	82.4	381	14.9	22	N
25	4.6	10.7	33.6	80.1	25.1	31.3	16.6	1.8	77.9	380	11.7	20	N
29	4.32	12.1	38.2	90.8	27.5	31.2	14.2	2.1	76.8	325	12.6	24	N
32	4.78	10.2	32.1	80.1	24.1	31.5	16.4	1.9	59.6	370	17.1	16	N
22	3.61	12.3	37.5	89.2	28	32.1	16.1	1.8	55.8	365	15.8	15	N
28	4.05	12	37	88.7	31.9	31.6	13.1	1.7	81.2	381	15.4	21	N

25	4.16	12.2	37.9	92.4	30.8	32.1	12.7	1.9	56.7	380	18.4	15	N
26	4.1	8.6	27	78.3	31.4	21.1	13.5	1.8	61.2	325	19.6	19	N
27	3.83	10.4	30.9	82.5	26.9	32.3	18.2	1.7	94	385	12.9	24	N
32	3.71	10.2	31	77.2	31	33.2	13.8	2	88	389	13.8	23	N
22	4.27	12.4	35.6	92.4	28.6	31.9	16.2	1.9	38.7	389	14.5	10	N
21	4.11	10.1	31	72.2	29.4	32.1	13.8	1.8	42.5	390	12.5	11	N
29	4.09	11.1	33.8	83	30.8	30.2	14.5	2.1	59.7	380	13.1	16	N
28	4.09	10.7	32.1	78.2	29.8	30.7	14.3	1.9	69.9	325	11.4	21	N
32	3.98	10.1	30.5	80.4	21.6	31.6	17.1	1.8	39.8	370	12.7	11	N
28	4.55	10.5	33.2	80.9	25.9	30.9	14.5	1.7	41.2	365	12.6	11	N
27	4.01	11.2	34.2	83.2	28.7	31.9	14.3	1.9	65.2	381	17.1	18	N
32	4.17	10.4	35.3	82.6	27.3	31.2	15.2	1.8	64	380	15.8	17	N
30	3.74	10.2	33.3	79.1	30.7	31.8	15.5	1.7	82	325	11.4	25	N
31	4.3	11.3	33.9	80.1	24.6	30.5	14.8	2	57.2	370	18.4	15	N
37	4.2	10.1	30	80.5	26.5	31.3	14	1.9	61	365	14.9	17	N
28	3.78	11.2	33.4	81.2	21.9	30.2	19.7	1.8	64.3	381	11.7	17	N
29	4.1	10.7	33.2	82	24.7	30.7	17.5	2.1	76	380	12.6	20	N
27	4.5	11.5	35.1	83.1	28	31.6	14.4	1.9	82.4	325	17.1	25	N
26	4.7	10.2	31.5	80.6	23.8	30.9	17.7	1.8	54	385	15.8	14	N
24	5.12	11.2	34	80.9	26.8	31.9	14.8	1.7	85.6	389	15.4	22	N
28	4.25	11.3	34	80	24.9	31.2	18.2	1.9	82.4	389	18.4	21	N
29	4.07	11.4	34.6	83	27.6	31.8	14.1	1.8	77.9	390	19.6	20	N
30	4.9	8.6	27	74.4	22.7	30.5	14.9	1.7	76.8	380	10.9	20	N
28	4.6	10.4	31	80.1	25.1	31.3	16.6	2	62.4	325	13.8	19	N
27	4.32	10.2	32	74.4	27.5	31.2	14.2	1.9	38.9	370	14.5	11	N
21	4.78	11.3	35.1	80.1	24.1	31.5	16.4	1.8	85.6	365	12.5	23	N
22	3.61	10.1	31.5	75.6	28	32.1	16.1	2.1	82.4	381	13.1	22	N
20	4.05	11.1	35.1	79.8	21.6	31.6	13.1	1.9	77.9	380	11.4	20	N

22	4.16	10.7	33.1	80.4	25.9	32.1	12.7	1.8	76.8	325	12.7	24	N
29	4.1	10.1	30.7	78.3	26.2	21.1	13.5	1.7	59.6	370	14.9	16	N
27	3.83	10.5	30.9	82.5	25.4	32.3	18.2	1.9	55.8	365	11.7	15	N
24	3.71	11.2	33.8	77.2	26.4	33.2	13.8	1.8	81.2	381	10.1	21	N
25	4.27	10.4	30.2	79.6	24.6	31.9	16.2	1.7	56.7	380	14.5	15	N
26	4.11	10.2	32	72.2	26.5	32.1	13.8	1.8	61.2	325	12.5	19	N
30	4.09	11.3	33.5	80.5	21.9	30.2	14.5	1.9	94	385	11.7	24	N
33	4.09	10.1	32.1	82.4	24.7	30.7	14.3	2.1	88	389	11.4	23	N
26	3.98	11.2	35.1	81.4	28	31.6	17.1	1.9	82.4	389	12.5	21	N
25	4.55	10.7	32.7	80.9	23.8	30.9	14.5	2.1	77.9	390	10.4	20	N
35	4.01	11.5	34.2	80	26.8	31.9	14.3	2.3	76.8	389	11.1	20	N
34	4.17	10.2	31.8	82.6	24.9	31.2	15.2	1.9	59.6	386	12.2	15	N
29	3.74	11.2	33.3	80.1	26.4	31.8	15.5	2.1	55.8	390	10.1	14	N
28	3.85	11.3	33.4	80.1	22.7	30.5	15.1	1.8	81.2	387	12.6	21	N
27	4.09	11.4	33.8	82.1	26.1	31.3	12.8	3.2	56.7	392	11.2	14	N
22	4.27	10.3	30.7	81.2	27.5	33.6	12.9	2.1	61.2	386	10.8	16	N
20	4.05	12	34.3	81.1	27.8	33	13.4	2.3	72.4	385	14.4	19	N
23	4.04	10.8	34.3	78.6	26.4	34.7	13	2.4	65.3	387	11.1	17	N

3- Pregnant women without iron therapy

Age	RBC	Hb	HCT	MCV	MCH	MCHC	RDW	Retics	S.Iron	TIBC	S.Ferritin	TS	Hb electrophoresis
31	3.9	12.1	36	92.3	29.2	31.7	13.9	0.5	35.2	270	12.4	13	N
28	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.7	66.2	390	7.8	17	N
29	3.78	8.1	25.6	62.6	18.4	29.5	20.3	0.2	52.2	395	8.4	13	N
33	3.75	8.8	27.2	62.9	19.6	30.1	21.8	0.3	61.3	391	9.1	16	N
24	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.1	51.2	389	9.2	13	N
33	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.5	59.7	381	8.9	16	N
31	3.65	9.9	30.2	74.6	25.7	30.8	17.5	0.7	53.4	395	8.1	14	N
30	4.22	11.1	31.6	74.9	24.4	32.6	15.9	0.2	62.5	388	12.7	16	N
35	3.87	9.4	27.7	71.5	24.3	30.9	18.3	0.3	66.2	391	9.1	17	N
39	4.1	10.2	31.8	72	23.1	32.1	18.1	0.1	52.2	387	10	13	N
38	3.89	9.7	30.7	80.2	24.9	30.6	18.4	0.5	61.3	388	9.5	16	N
37	4.3	12.8	39.9	91.9	29.5	32.1	13.9	0.7	51.2	279	12.4	18	N
25	3.78	9.3	28.4	68	20.9	30.7	16.5	0.2	59.7	391	9.4	15	N
29	3.5	9.2	27.9	79.9	26.3	33	15.3	0.3	53.4	389	9.8	14	N
28	4.04	9.9	31	79.5	24.5	30.8	16.5	0.1	62.5	387	9.8	16	N
31	3.59	9	28.1	81.2	25.1	30.8	17.2	0.5	52.2	395	9.1	13	N
30	4.23	9.8	31	73.3	23.3	31.6	19.1	0.7	66.2	389	9.6	17	N
22	3.77	9.8	31.3	61.3	19.2	31.3	20.4	0.2	52.2	395	9.7	13	N
35	4.03	11.1	34.1	82.7	26.8	31.7	14.8	0.3	61.3	379	14.3	16	N
36	3.9	12.1	36	92.3	29.2	31.7	13.9	0.1	51.2	270	12.4	19	N
34	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.5	59.7	390	12.2	15	N
35	3.78	8.1	25.6	62.6	18.4	29.5	20.3	0.7	53.4	395	8.4	14	N
36	3.75	8.8	27.4	62.9	19.6	30.1	21.8	0.2	62.5	391	9.1	16	N
25	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.3	66.2	389	9.2	17	N
27	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.1	52.2	381	8.9	13	N

31	3.65	9.9	30.2	74.6	25.7	30.8	17.5	0.5	61.3	395	8.1	16	N
33	4.22	10.3	31.6	74.9	24.4	32.6	15.9	0.7	51.2	388	9.8	13	N
35	3.87	9.4	27.7	71.5	24.3	30.9	18.3	0.2	59.7	391	12.1	15	N
36	4.1	10.2	31.8	72	23.1	32.1	18.1	0.3	53.4	387	10	14	N
31	3.89	9.7	30.1	81.4	24.9	30.6	18.4	0.1	62.5	388	9.5	16	N
28	4.3	12.8	39.9	91.9	29.5	32.1	13.9	0.5	66.2	279	12.4	24	N
29	3.78	9.3	29.6	68	20.9	30.7	16.5	0.7	52.2	391	9.4	13	N
27	3.5	9.2	27.9	79.9	26.3	33	15.3	0.2	61.3	389	9.8	14	N
24	4.04	9.9	31	79.5	24.5	30.8	16.5	0.3	51.2	387	9.8	13	N
33	3.59	9	28.4	81.2	25.1	30.8	17.2	0.1	59.7	395	9.1	15	N
30	4.23	9.8	31	73.3	23.3	31.6	19.1	0.5	53.4	389	9.6	14	N
39	3.77	9.8	31.3	61.3	19.2	31.3	20.4	0.7	62.5	395	9.7	16	N
38	4.03	10.8	34.1	82.1	26.8	31.7	14.8	0.2	66.2	379	12.4	17	N
35	3.9	12.1	36	92.3	29.2	31.7	13.9	0.9	52.2	270	12.4	19	N
31	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.7	61.3	390	7.8	16	N
30	3.78	8.1	25.1	62.6	18.4	29.5	20.3	0.5	51.2	395	8.4	13	N
36	3.75	8.8	26.4	62.9	19.6	30.1	21.8	0.7	59.7	391	9.1	15	N
35	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.2	53.4	389	9.2	14	N
34	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.3	62.5	381	8.9	16	N
21	3.65	9.9	30.2	74.6	25.7	30.8	17.5	0.1	52.2	395	8.1	13	N
22	4.22	11.4	32.2	74.9	24.4	32.6	15.9	0.5	66.2	388	14.2	17	N
27	3.87	9.4	27.7	71.5	24.3	30.9	18.3	0.7	52.2	391	9.1	13	N
30	4.1	10.2	31.8	72	23.1	32.1	18.1	0.2	61.3	387	12.4	16	N
35	3.89	9.7	30.8	81.4	24.9	30.6	18.4	0.3	51.2	388	9.5	13	N
29	4.3	12.8	39.9	91.9	29.5	32.1	13.9	0.1	59.7	279	12.4	21	N
31	3.78	9.3	29.6	68	20.9	30.7	16.5	0.5	53.4	391	9.4	14	N
28	3.5	9.2	27.9	79.9	26.3	33	15.3	0.7	62.5	389	9.8	16	N
31	4.04	9.9	31	79.5	24.5	30.8	16.5	0.2	66.2	387	9.8	17	N

35	3.59	9	28.7	81.2	25.1	30.8	17.2	0.3	52.2	395	9.1	13	N
25	4.23	9.8	31	73.3	23.3	31.6	19.1	0.1	61.3	389	9.6	16	N
24	3.77	9.8	31.3	61.3	19.2	31.3	20.4	0.5	51.2	395	9.7	13	N
32	4.03	11.2	34.1	84.5	26.8	31.7	14.8	0.7	59.7	379	12.3	16	N
38	3.9	12.1	36	92.3	29.2	31.7	13.9	0.2	53.4	270	12.4	20	N
26	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.5	62.5	390	7.8	16	N
36	3.78	8.1	25	62.6	18.4	29.5	20.3	0.7	66.2	395	8.4	17	N
26	3.75	8.8	26.5	62.9	19.6	30.1	21.8	0.2	52.2	391	9.1	13	N
35	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.3	61.3	389	9.2	16	N
34	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.1	51.2	381	8.9	13	N
25	3.65	9.9	30.2	74.6	25.7	30.8	17.5	0.5	59.7	395	8.1	15	N
26	4.22	10.3	31.6	74.9	24.4	32.6	15.9	0.7	53.4	388	16.2	14	N
39	3.87	9.4	27.7	71.5	24.3	30.9	18.3	0.2	62.5	391	9.1	16	N
38	4.1	10.2	31.8	72	23.1	32.1	18.1	0.3	66.2	387	13.2	17	N
31	3.89	9.7	30	81.4	24.9	30.6	18.4	0.1	52.2	388	9.5	13	N
30	4.3	12.8	39.9	91.9	29.5	32.1	13.9	0.5	61.3	279	12.4	22	N
25	3.78	9.3	28.4	68	20.9	30.7	16.5	0.7	51.2	391	9.4	13	N
22	3.5	9.2	27.9	79.9	26.3	33	15.3	0.2	59.7	389	9.8	15	N
28	4.04	9.9	30	79.5	24.5	30.8	16.5	0.3	53.4	387	9.8	14	N
31	3.59	9	28.3	81.2	25.1	30.8	17.2	0.1	62.5	395	9.1	16	N
32	4.23	9.8	31	73.3	23.3	31.6	19.1	0.5	52.2	389	9.6	13	N
25	3.77	9.8	31.3	61.3	19.2	31.3	20.4	0.7	66.2	395	9.7	17	N
26	4.03	10.8	34.1	84.5	26.8	31.7	14.8	0.2	52.2	379	15.5	14	N
31	3.9	12.4	36	92.3	29.2	31.7	13.9	0.5	61.3	270	12.4	23	N
28	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.7	51.2	390	7.8	13	N
26	3.78	8.1	25.1	62.6	18.4	29.5	20.3	0.2	59.7	395	8.4	15	N
36	3.75	8.8	27	62.9	19.6	30.1	21.8	0.3	53.4	391	9.1	14	N
34	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.1	62.5	389	9.2	16	N

25	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.5	66.2	381	8.9	17	N
27	3.65	9.9	30.2	74.6	25.7	30.8	17.5	0.7	52.2	395	8.1	13	N
37	4.22	12.1	35.9	83.1	28	32.6	15.9	0.2	61.3	388	12.3	16	N
35	3.87	9.4	27.7	71.5	24.3	30.9	18.3	0.3	51.2	391	9.1	13	N
28	4.1	12	35	83	27.1	32.1	18.1	0.1	59.7	387	15.2	15	N
29	3.89	9.7	30.6	81.4	24.9	30.6	18.4	0.5	53.4	388	9.5	14	N
24	4.3	12.8	39.9	91.9	29.5	32.1	13.9	0.7	67	279	12.4	24	N
35	3.78	9.3	28.3	68	20.9	30.7	16.5	0.2	58	391	9.4	15	N
31	3.5	9.2	27.9	79.9	26.3	33	15.3	0.3	61	389	9.8	16	N
30	4.04	9.9	30.1	79.5	24.5	30.8	16.5	0.1	62.3	387	9.8	16	N
36	3.59	9	28.5	81.2	25.1	30.8	17.2	0.5	62	395	9.1	16	N
35	4.23	9.8	31	73.3	23.3	31.6	19.1	0.7	43.2	389	9.6	11	N
28	3.77	9.8	31.3	61.3	19.2	31.3	20.4	0.2	68.4	395	9.7	17	N
29	4.03	12.1	34.1	84.5	27.2	31.7	14.8	0.5	69.4	379	13.1	18	N
30	3.9	13	36	92.3	29.2	31.7	13.9	0.7	66.7	270	12.4	25	N
24	2.43	7.5	23.4	71.5	25.8	28.7	15.1	0.2	62.3	390	7.8	16	N
28	3.78	8.1	25	62.6	18.4	29.5	20.3	0.3	64.2	395	8.4	16	N
31	3.75	8.8	27.4	62.9	19.6	30.1	21.8	0.1	58	391	9.1	15	N
37	3.71	9.2	28.3	67.4	21.9	32.5	19.5	0.5	61	389	9.2	16	N
33	2.81	8.4	25.2	74.6	25.7	29.8	14.9	0.7	63.4	381	8.9	17	N
36	4.21	11.4	33.3	79.1	25.4	32.1	13.8	0.2	65.6	389	12.2	17	N
30	3.99	11.5	32.6	81.7	26.1	31.9	13.9	0.3	59.8	379	13.3	16	N
25	3.6	11.3	30.6	85	28.3	33.3	12.4	0.1	64.7	270	14.2	24	N
29	4.11	11.2	33.2	80.8	25.8	31.9	13.1	0.5	51.2	390	12.8	13	N
28	3.77	6.7	21	62.1	17.8	28.6	16.1	0.7	59.7	395	12.4	15	N
37	3.76	6.8	21.6	62.2	18.1	29.1	16.3	0.2	53.4	391	9.4	14	N
25	4.16	10.8	33.3	80	26	32.4	11.3	0.3	66.2	389	13.1	17	N
36	4.42	11.8	33.5	72.9	23.3	32	12.8	0.1	68.1	381	12.4	18	N

21	4.28	8.8	27.4	65.9	20.6	31.2	13.4	0.5	63.1	395	9.1	16	N
32	5.26	11.4	34.9	66.3	20.7	31.2	13	0.7	64	388	12.2	16	N
33	4.57	8.1	25.6	60.4	17.7	29.3	14.8	0.2	70.1	387	9.7	18	N
35	3.67	10.1	31.2	85	27.5	32.4	12.9	0.9	62.3	388	12.1	16	N
29	4.07	9.9	30.4	74.7	24.3	32.6	12.4	0.7	65.8	279	12.4	24	N
39	4.03	8.4	25.4	67.7	20.8	30.8	15.1	0.5	66.3	391	7.8	17	N
30	3.9	11.5	33.4	85.6	27.4	32	12.1	0.7	62	389	12.5	16	N
28	4.26	11.4	33.1	74.2	23.7	32	13.2	0.2	66.2	387	12.4	17	N
37	4.4	9.9	31.4	71.4	22.5	31.5	14	0.3	61.7	389	9.5	16	N
27	3.25	10.1	31.4	96.6	31.1	32.2	19.2	0.1	61.3	395	12.4	16	N
30	4.2	10	31.3	74.5	23.8	31.9	12.9	0.5	62.7	379	12	16	N
28	4.1	10.3	31.5	76.8	25.1	32.7	11.8	0.7	67.6	270	13.1	25	N
21	3.59	10.5	31.7	88.3	29.2	33.1	11.8	0.2	82.4	390	12.4	21	N
33	5.19	8.3	26.3	56.5	16	28.3	17.4	0.3	66.7	395	9.8	17	N
36	4.07	11.2	32.9	80.8	26.3	32.5	15.7	0.1	76.5	391	12.1	20	N
38	3.82	11.3	32.5	85.1	28.5	33.5	12.4	0.5	75.4	389	13.2	19	N
34	4.25	11.5	32.7	76.9	24.9	32.4	14	0.7	66.8	381	15.1	18	N
25	4.39	11.7	34.1	71.5	23.9	33.4	15	0.2	79.2	395	12.4	20	N
21	3.24	9.5	28.5	88	29.3	33.3	11.8	0.3	63.5	388	9.4	16	N
28	3.87	9	28.4	73.4	23.3	31.7	14.7	0.1	65.7	391	9.8	17	N
25	3.96	10.1	31.4	79.3	25.5	32.2	13.4	0.5	75.8	389	12.2	20	N
26	3.33	9.8	29.5	88.6	29.4	33.2	12.8	0.7	65.6	381	9.1	17	N
35	4.4	10.2	31.9	72.5	23.2	32	12.1	0.2	82.3	395	12.4	21	N
33	3.9	9	28.3	72.6	23.1	31.8	11.6	1	64.4	388	9.1	16	N
30	4.16	10.5	32.5	78.1	25.2	32.3	15.8	0.7	65.6	391	9.2	17	N
31	3.78	8.1	25.8	68.3	21.4	31.4	14.5	0.5	70.4	387	12.1	18	N
32	4.57	11.3	32.7	71.6	22.5	31.5	15.2	0.6	80.1	388	12	21	N
36	3.91	11.1	34.2	79.3	26.1	32.9	11.6	0.9	62.3	279	12.1	22	N

35	3.86	11.2	33.2	83.4	27.7	33.2	12.7	1.1	61	391	13.2	16	N
21	2.79	6.8	21.2	76	24.4	32.1	15.8	0.7	73.1	389	8.9	19	N
36	2.82	6.9	21.3	75.5	24.5	32.4	15.5	0.9	68	387	8.1	17	N
35	4.38	8.8	26.5	65.8	20.1	30.6	18.3	0.7	69.5	395	9.8	17	N
34	4.39	8.7	27.2	66.1	19.8	30	18.5	0.5	62.1	389	9.1	16	N
25	3.35	11.2	33.2	89.6	30.7	34.3	11.3	0.6	65.4	389	12.5	17	N
26	4.33	11.1	33.3	76.9	24.9	32.4	14.3	0.9	75.7	379	14.5	20	N
32	3.69	11.4	34.1	78.3	27.9	35.6	13.5	1.1	75.1	270	15	28	N
32	3.89	9.5	29	74.6	24.4	32.8	13.4	0.7	63.7	390	9.4	16	N
36	3.41	9.2	28.5	78.1	27	32.3	15.2	0.9	65.6	395	9.8	17	N
25	2.81	7.6	23.3	82.9	27	32.6	15	1.5	68.4	391	9.8	17	N
28	3.9	10.7	33.2	82.4	27.4	32.2	18.1	3.1	71.4	389	12.3	18	N
29	3.95	10.9	32.8	82	27.6	33.2	13.2	1.1	65.7	381	12.1	17	N

Appendix 4



Cell dyn 1700 (for complete hemogram)