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Physiological Changes in Iron and Blood Parameters during Different Pregnancy Trimesters in Pregnant Women in Baghdad

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Abstract

This work was carried out for four months in the province of Baghdad including many hospitals in Baghdad during the 1st of November 2016 until 28 February 2017. 120 pregnant women included in the present study ranged in age from 14 - 42 years were randomly selected. Pregnant women were divided into three groups according to the different periods of pregnancy every trimester include 40 pregnant women divided to 20 pregnant women with anemia and 20 without anemia after taking a blood film and hemoglobin as a good adoption indicator for diagnosis of anemia, also, history taken by previously diagnosis and by physical examination. Result of the three trimesters in indicators the blood of pregnant women with or without anemia, showed that the first trimester all of the parameters of indicators the blood (Hb, Hct, MCV, MCH and MCHC) were decreased in women with anemia compared with women without anemia, also in second and third trimester decreased. However, in second trimester the values of Hb, Hct, MCV, MCH and MCHC decreased in women with anemia on the values of the first trimester. On the other hand a values of indicators the blood in a third trimester less than the second trimester. Indicator of iron (SI, TIBC, TS and SF) for women with anemia in first trimester was less than from women without anemia. However, in second trimester the values of SI, TIBC, TS and SF decreased in women with anemia on the values of the first trimester. Father more, the values of indicator of iron in a third trimester less than in the second trimester. When comparison in blood parameters (Hb, Hct, MCV, MCH and MCHC) between 1st, 2nd, and 3rd trimester of pregnancy women noticed that the all parameters decreased gradually from the first trimesters to second to third trimester. Iron deficiency marked increase was famous in pregnant women in the second and third trimester of pregnancy, due to high fetal and placenta growth rates and development of red cell mass mother. Thus anemia affects up to 70% of pregnant women. Blood indicators RBC, RDW, platelet and MPV for all pregnant women in the first trimester was decreased in women with anemia compared with women without anemia in all parameters of this, also in second and third trimester. Furthermore, in second decreased from first, also in third less that from second. White blood cell and their various types (NEU, LYM, Mono, ESO and BASO) were impressed with the pregnant women with anemia in first and second trimesters WBC and NEU% increased but LYM%, Mono%, ESO% and BASO% decreased. However, in the third trimester WBC, NEU% and BASO% decreased but LYM%, Mono%, and EOS% increased. The results of the study found out that the is a clear correlation between anemia in pregnant women and some social factors, such as: occupation, monthly income and maternal education.

Keywords: Physiological changes, Blood parameters, pregnant women

الخلاصة

تم تنفيذ هذا العمل لمدة أربعة أشهر في العديد من المستشفيات في محافظة بغداد خلال الفترة من 1تشرين الثاني 2016 حتى 28 شباط 2017.تم اختيار 120 من النساء الحوامل في هذه الدراسة عشوائيا تراوحت أعمارهم من 14 - 42 سنة .تم تقسيم النساء الحوامل إلى ثلاث مجموعات وفقا لفترات الحمل المختلفة كل ثلاثة أشهر تشمل 40 امرأة حامل مقسمة إلى 20 امرأة حامل مع فقر الدم و 20 دون فقر الدم بعد أخذ مسحة الدم والهيموغلوبين كمؤشر جيد لتشخيص فقر الدم،وكذلك تاريخ المريض والفحص الجسدي. وأظهرت النتائج للنساء الحوامل المصابات بفقر الدم أو بدونه في المراحل الثلاثة أن مؤشرات الدم (Hb، Hct، MCV، MCH،MCHC) للثلث الأول قد انخفضت في النساء المصابات بفقر الدم مقارنة بالنساء دون

فقر الدم، وأيضا في الثلث الثاني والثالث انخفضت .ومع ذلك في الأشهر الثلاثة الثانية انخفضت قيم (Hb، Hct، MCV، MCHC،MCH) في النساء مع فقر الدم عن قيم الأشهر الثلاثة الأولى .من ناحية أخرى فإن قيم مؤشرات الدم في الثلث الثالث من الحمل أقل من الثلث الثاني. وكان مؤشر الحديد (SI، TIBC، SF and TS) للنساء المصابات بفقر الدم في الثلث الأول من الحمل أقل من النساء دون فقر الدم .ومع ذلك في الأشهر الثلاثة الثانية انخفضت قيم (SI، TIBC ، TS and SF) في النساء مع فقر الدم عن قيم الأشهر الثلاثة الأولى .وكذلك قيم مؤشر الحديد في الثلث الثالث من الحمل أقل من الثلث الثاني.عند المقارنة في معايير الدم (Hb، Hct، MCV، MCH،MCHC) بين الثلث الاول والثاني والثالث الثلث من الحمل لوحظ أن جميع المعلمات انخفضت تدريجيا من الثلث الأول إلى الثاني إلى الثلث الثالث. وقد انخفضت مؤشرات الدم (RBC،RDW ، الصفائح الدموية و MPV لجميع النساء الحوامل في الأشهر الثلاثة الأولى في النساء مع فقر الدم مقارنة مع النساء دون فقر الدم ، وأيضا في الثلث الثاني والثالث .وعلاوة على ذلك انخفضت أكثر في المرحلة الثانية عن قيم المرحلة الأولى، وأيضا في المرحلة الثالثة أقل من المرحلة الثانية. وان خلايا الدم البيضاء وأنواعها المختلفة (Eso, Mono,Lym (Baso and للنساء الحوامل مع فقر الدم في الثلث الأول والثاني زادت Neu% ولكن Mono%, Baso% Eso% and Lym% انخفضت. ومع ذلك، في الثلث الثالث Lym%،Baso% انخفضت ولكن Lyu%، Eso%،Mono% زيادة .ومن نتائج الدراسة وجدت أن هناك علاقة واضحة بين فقر الدم في النساء الحوامل وبعض العوامل الاجتماعية مثل الدخل الشهري وتعليم الأمهات وهل الام ربة منزل ام موظفة.

Introduction

Accompanying pregnancy physiological changes that result in an increase in blood plasma and red blood cells and low concentration of nutrients associated with protein and micronutrient like iron, folic acid and vitamin B12 these physiological changes as a result of malnutrition, which leads to shortages of basic elements described anemia [1]. Iron is one of the important requirements for the composition of hemoglobin, myoglobin and cytochrome oxidase and other enzymes [2]. Pregnancy induces a number of changes in the normal physiology, either directly or indirectly. Important of these changes are those which alter the hematological parameters. Plasma expansion and hemodilution during pregnancy contribute to majority of these changes. The most frequent hematologic complication during pregnancy is anemia [3]. WHO standard for anemia in women is Hb less than 120 gm /L and less than 110 gm /L in pregnant women due to physiological anemia [4]. Anemia during pregnancy due to increased demand for iron and other vitamins due to the physiological burden of pregnancy [5]. Anemia is the most prevalent nutritional deficiency during pregnancy [6]. Definition of iron deficiency anemia is generally as defined as a decrease in the oxygen carrying capacity of the blood and considered as the main cause of microcytic hypochromic anemia. This reduces the hemoglobin and volume red blood (hematocrit) or the number of erythrocytes [7]. Hemoglobin drop below normal levels in some cases up to 10 g/dl [8]. Iron deficiency may increase the risk of maternal infection, and hemoglobin decrease may cause a low degree of chronic hypoxia that may address maternal and fetal stress, as it can increase oxidation resulting in damage to erythrocytes and feto-placenta unit [9]. In the present study, we identified risk factors associated with severe anemia among a large sample of pregnant women in many hospital of Baghdad. The aim of our current study is to: Determine the effect of pregnancy on blood parameters and compare blood parameters at different stages of pregnancy.Improve pregnancy outcome are dependent on the quality and quantities of hematological indices. To determine proportion of anemia among pregnant women, to identify factors associated with the anemia.

Materials and Methods

Samples Collection: The study included 120 pregnant women of Baghdad province, ranged in age from (14_42 years) were randomly selected for this study. Pregnant women were divided into three groups according to the different periods of pregnancy, As follows: The first trimester of pregnancy included were 40 pregnant women, (20 pregnant women with anemia and 20 pregnant women without anemia). The second trimester of pregnancy of pregnancy included were 40 pregnant women, (20 pregnant women with anemia and 20 pregnant women without anemia). Third quarter of pregnancy included were 40 pregnant women, (20 pregnant women with anemia and 20 pregnant women without anemia). It was withdrawn model of venous blood from pregnant women in different periods of pregnancy and ages randomly during

the period between 8:30AM to 12 PM. In present study have been getting samples from the department of laboratory in hospitals and health centers deployed in the province of Baghdad, including Baghdad teaching hospital, AL-Elweya hospital , AL-Karama hospital, Fatima AL-Zahra hospital, AL-Karkh hospital, AL-Kadhimiya hospital, and a health center in Al- Doura, a health center in Al-Shaab. During the period from the 1st of the November 2016 until 28 February 2017. After determine of Hb to know the pregnant with anemia or without. If the pregnant Hb under 10g/dL is anematic women and blood film . Present study attended many of the glass tubes containing anti-clotting substance-sized (5 mL) glass tube holds number for each person to a similar number to which questionnaire paper, which was included pregnant women information for examination the same person. Then it was withdrawing (5 mL) of venous blood from the women by used disposable syringes (5 mL) and were divided blood into two parts (2.5 mL) one part placed in tube containing anticoagulant substance (EDTA) ,and then close it the movement of thin to mix the blood with article anticoagulant and then were taken blood for the purpose of conducting blood tests, and another part (2.5 mL) was placed in the tube does not contain anticoagulant, present study left at room temperature and then placed in a centrifuge for 10 minutes at the speed of 3000 cycles/minute for get on serum, and then put the resulting serum in ependroff tubes and stored in deep freeze at a temperature -20˚C, till examination for biochemical assay. Statistical analysis :Analysis of the results statistically ensure the use of the test t samples for independent comparison of averages, standard error of the mean standard error of mean (SEM), the coefficient coefficient, and the use of variation analysis ANOVA (One way) . Each data were analyzed using SPSS statistical software [10].

A. Measurement of blood test

1- Measurement of hemoglobin (Hb) (g/dL).
2-Calculate prepare the red blood cells (RBCS) (106/mm3)
3-Calculate the percentage of the packed red blood cells volume (PCV%) (Hct).
4-Calculate the volume of red blood cells (MCV) (fL).
5-Calculation the weight of hemoglobin (MCH) (pg).
6-Calculation the average concentration of hemoglobin (MCHC) (g/dL).
7-Calculation a measurement of the variation in RBC cell size (RDW) (%).
8- Calculate a prepare platelet blood (103/mm3).
9. Calculate a prepare of white blood cells WBCs (109/L).
10-Calculated of the average size of mean platelet volume (MPV).
11-Examinationof blood film .

B-Measurement of biochemical test

1-Measurement the level of serum iron (SI) (µmol/L) [11],[12].
2-Measurement of the total iron binding capacity (TIBC) (µmol/L) [13].
3-Measurement the level of ferritin serum (FS) (ng/mL)

Results and Discussion

Blood Film : Blood smear or (film) is one of the world's most widely and frequently used tests. Blood film very useful tool in hematological assessment (RBCs, WBC estimates, platelet estimates) and to study the morphology of RBC during pregnancy to appear the number of anemic pregnant women , also to knowledge of the type of anemia, the most common one was microcytic and hypochromic . The study showed test blood film of every pregnant (120 women) in these experiment to find out pregnant women positive anemia and non-positive before every test. However, to determine the which of the type of anemia from the fourth types.

In this study of peripheral smear is a good indicator for diagnosis of anemia. Also, by history taken from pregnancy and physiological examination (1- pallor-

inconjuctiva of eye, pal mar creases, mucous membrane month. 2- history of headache and fatigue. 3 - angular inflammation of the mouth (erosion of tenderness and swelling of the corners of the mouth). 4. The tongue may also be smooth, shiny and inflamed, called inflammation of the tongue. Determines anemia before classification the pregnant women.

Chang of Physiological Parameters in Pregnancy

Physiology section were the pregnancy period is divided into for 40 weeks to three stages each stage is called trimester and lasts three months. The first third begins from 0-13 weeks, it starts from the beginning of the first day of another occurrence of menstrual cycle. Second third begins from 13 to 26 weeks, and the third begins from 26 to 40 weeks of pregnancy period [14].

First Trimester of Pregnancy

The hematological parameters according to first trimester of pregnancy women is present in Table 1, Hemoglobin value in serum of blood of pregnant women without anemia (control) was (12.30±0.186 g/dL) that increased highly significantly ($p < 0.01$) from women with anemia (10.09±0.271 g/dL) . Also, there was highly significant difference ($p < 0.01$) in Hct % between women without anemia and women with anemia that was (39.59±0.708) vs (32.88±0.650)% respectively. On the other hand, depending on the data in Table 1, the mean value of MCV was (87.05±0.871 fL) vs (85.69±1.297 fL) in pregnant women without and with anemia, respectively. In the current study, the data obtained demonstrated a decreasing in each of MCH and MCHC means in women with anemia group as compared with women without anemia. The data present verified MCH means ± SD in women without anemia and women with anemia were (34.45±0.606) and (33.64±0.663) pg also, MCHC mean were (30.55±0.357) and (28.88±0.384) g/dL respectively.

Results of the present study were in agreement with that recorded by [15], who found that the concentration of Hb began to decrease from a 10-12 week of pregnancy period as a result of the increase of blood plasma, which leads to anemia. Also, ferritin serum decreased clearly between 12-24 weeks from pregnancy period, that is resulted from iron consumption when a maternal red blood cell mass increased [16]. Also, transferrin saturation decreased clearly with iron and decreased in a volume of RBC, these indicators show the status of decrease in iron [17].

Table 1: Comparative in indicators the blood of women without anemia and women with anemia in the first trimester of pregnancy.

Parameters	Women without anemia n = 20	Women with anemia n = 20
Hb (g/dL)	12.30±0.186	10.09±0.271**
Hct (%)	39.59±0.708	32.88±0.650**
MCV (fL)	87.05±0.871	85.69±1.297
MCH (pg)	34.45±0.606	33.64±0.663
MCHC (g/dL)	30.55±0.357	28.88±0.384

All results are presented as mean ± SD values (M±SD)

** significant at $p < 0.01$

Najlaa and Iqbal [18], reported that the Hb value 11.26 g/dL in pregnant was lower than non pregnant , this also agree with Luay [19], who reported that a Hb and Hct decreased significantly in women with anemia compared with women without anemia in first trimester of pregnancy . However, MCV, MCH and MCHC were decreased but not significantly in women with anemia.

From the parameters in Table 1, we can discover anemia early during the first trimester in the popular pre-natal clinics[20]. Hb and Hct values from the basic tests to detect about anemia, as well as other special examinations of anemia such as microcytic anemia and

hypochromic anemia from CBC with indication of depleting the iron stores, such as a decrease in serum iron level, the rise in TIBC, decline in serum ferritin level with increase in protoprofirin RBC [17].

In the study of Abeer [21], showed moderate anemia in 38.8%, severe anemia in 21.3%, mean hemoglobin 9.2, mean corpuscular volume (MCV)109, mean serum ferritin 48.7, serum iron 104 and total iron binding capacity 458 in pregnant women in Ibn-Albalady hospital in Baghdad. Another study done in Algeria by Demmouche [22], found the mean Hb level to be Hb 9 g/dL and MCV 75.7, these results higher than the standard value of 78, this may indicate low dietary intake.

In the first trimester Abdul Rahman [23], showed that PCV and hemoglobin in pregnant women were less than control group of women in Tikrit Teaching Hospital during pregnancy.

Other studies had conducted worldwide such as that of Klebanoff et al [24]., in Egypt had Hb levels below 8g/dL , Also, in Jordan pregnant women included urban and rural areas the Hb level was less than 11 g/dl [25]. The mean Hb level of pregnant women in the 1st, 2nd and 3rd trimester was 11, 10.4 and 10.4 g/dL, respectively [26] in Iraq . From West Indies the mean Hb was 11.7 g/dL from an urban area[27].

The results of Table 2 show that the mean ±SD in the 1st trimester is highly significant ($p< 0.01$) difference in both SI, TIBC, TS and SF. The average of SI in women without anemia (12.51±1.323) vs (8.87±1.646) µmol/L in women with anemia . TIBC values in women without anemia (85.51±1.323) µmol/L were higher than in women with anemia (74.48±9.342) µmol/L while the ratio of TS is lower in women without anemia (16.83±1.924) % from the women with anemia (10.51±1.323) %. But SF in women without anemia was (34.25±1.25) vs (18.22±1.092) ng/mL in women without anemia.

Table 2: Comparative in indicators of iron (SI, TIBC, TS and SF) for women without anemia and women with anemia in the first trimester of pregnancy .

Parameters	Women without anemia n= 20	Women with anemia n = 20
SI (µmol/L)	12.51±1.323	8.87±1.646**
TIBC(µmol/L)	85.51±1.323	74.48±9.342**
TS(%)	16.83±1.924	10.51±1.323 *
SF(ng/mL)	34.25±1.25	18.22±1.092 **

All results are presented as mean ± SD values
**P<0.01 * P<0.05

From these results in Table 2, we notice that hemoglobin began to decline from 10-12 weeks of interval load, as a result of the increase in the size of blood plasma which leads to anemia [15]. Also serum ferritin (SF) concentration began to decline clearly between the 12-24 weeks of pregnancy period, because of iron consumption expansion made in the maternal red blood cell mass [16].Also, the obvious drop in the transferrin saturation with iron with small size of RBC. All these indicators show that there is a lack of iron [17]. These results agree with Shu and Ogbodoa [20] in Nigeria who showed that the serum iron in the first trimester (10.92 µmol/L) was lower than in the second trimester (68.2 µmol/L).

Requirements In daily requirements, the requirement is 0.8 mg in iron in the first trimester, between 4 and 5 mg in the third trimester and> 6 mg in the third trimester [28].

The hematological parameters studied included (RBC, platelets, total WBC, neutrophils, monocytes, lymphocytes, eosinophil, basophil). Table 3, shows that there was highly significant ($p< 0.01$) increase in the mean value of RBC in women without anemia (111×106/mm3±0.108) due to first trimester of pregnancy compared with value of women with anemia (3.37×106/mm3±0.069). But, on the contrary red distribution width was less in women without anemia (12.575±0.248)% from average in women with anemia (13.475±0.490)% , it may be to make up the shortage in the number of RBC (Mohammed et

al., 2012) showed that there were a significant ($p< 0.05$) decrease in the mean of RBC in pregnant women (4.41×106/mm3) when compared with the mean control group (4.97×106/mm3).

Blood platelet was (244.75±13.625) and (212.75±11.651)×103/mm3in women without anemia and women with anemia respectively. Also, the main plate volume (MPV) in pregnant women with anemia (6.014±0.227) was higher than in women without anemia (6.006±0.224)×103/mm3.

Table 3: Comparison in blood indicators for all pregnant women in the first trimester of pregnancy (n = 40).

Parameters	Women without anemia n = 20	Women with anemia n = 20
RBC (10^6/mm^3)	111±0.108	3.376±0.069 **
RDW (%)	12.575±0.248	13.475±0.490
Platelet (10^3/mm^3)	244.75±13.625	212.75±11.651 *
MPV	6.006±0.224	6.014±0.227

All results are presented as mean ± SD values

** P<0.01 * P<0.05

White blood cells are responsible for body defense during pregnancy. WBC was reported to elevate white blood cells in this study Table 4 in women without anemia (7.939×109/L ±0.235) was less than in women with anemia (8.693×109/L±0.454) in first trimester, and the lymphocyte count was significantly ($p< 0.05$) higher in women without anemia (26.55%±0.989) compared to percentage in women with anemia (20.516%±2.364). But, the percentage was significantly ($p< 0.05$) in women without anemia (66.785%±2.159), (6.121%±0.277), (2.339%±0.379) and (4.00%±0.348) respectively less than in women with anemia (72.11%±2.809) (5.527%±0.516) (1.59%±0.221) and (3.132±0.420) respectively.

Table 4: Comparison in blood indicators among women without anemia and women with anemia in the first trimester of pregnancy.

Parameters	Women without anemia n = 20	Women with anemia n = 20
WBC ×109/L	7.939±0.235	8.693±0.454
NEU %	66.785±2.159	72.11±2.809 *
LYM %	26.55±0.989	20.516±2.364*
Mono %	6.121±0.277	5.5271±0.516
EOS %	2.339±0.379	1.59±0.221
BASO %	4.00±0.348	3.132±0.420

*P< 0.05

Human chorionic gonadotropin HCG rises up to a maximum concentration near the end of the first trimester and then drops to a low level thereafter ,HCG may inhibit antibody production whereas shown that HCG inhibited antibody formation of murine B cells [29]. Moreover B cells mediate humoral immunity by producing antibodies, and suppression of B lymphopoiesis during gestation [30]. In addition pregnancy represent stress state to women and stress has been found to be associated with lower immunoglobulin production [31].

Second Trimester of Pregnancy

Table 5 shows a highly significant ($p <0.01$) in Hb, Hct, MCH and MCHC but there was no significance in MCV in pregnant women in Baghdad province. Hemoglobin mean in pregnant women without anemia was (12.87 ± 0.191 g/dL) but, it decreased in women with anemia (9.74 ± 0.323 g/dL). On the other hand, Hct was (36.37 ± 0.118 %) in women without anemia, decreased significantly ($p <0.01$) to (31.52 ±1.064 %) in women with anemia.

However, MCV, MCH and MCHC values in women without anemia were (85.79 ± 0.485 fL), (29.78 ± 0.361 pg) and (33.92 ± 0.313 g/dL) respectively. Conversely there were (82.70 ± 1.523 fL), (26.6 ± 0.804 pg) and (30.05 ± 0.453 g/dL) respectively in women with anemia.

Table 5 : Comparative in indicators the blood of pregnant women without anemia and pregnant women with anemia in the second trimester of pregnancy.

Parameters	Women without anemia n = 20	Women with anemia n = 20
Hb (g/dL)	12.87±0.191	9.74±0.323**
Hct (%)	36.37±0.118	31.52±1.065**
MCV (fL)	85.79±0.485	82.70±1.523
MCH (pg)	29.78±0.361	26.6±0.804**
MCHC (g/dL)	33.92±0.313	30.05±0.453**

All results are presented as mean ± SD values

** significant at $p < 0.01$

Through the results showed in Table 5 it is believed that the most anticipated reasons for anemia in second trimester of pregnancy, is the decline in production of RBC, blood loss or low production. These factors result from the lack of basic items such as iron, vitamin B12 and folic acid or it may be related to a few basic elements for dietary deficiency, malabsorption, or bleeding, bone marrow suppression or disorders, hormone deficiency, chronic disease or infection leads to decrease of production or from hemolytic anemia which is associated with increased trashing cells [32]. These results agree with many results conducted to see the spread of lack of essential nutrients, from these studies[33]. In Indian town showed that the low intake of nutrients during pregnancy period may lead to the low concentration of micro-nutrients in the blood[33]. In another study conducted in China results pointed to the zinc and copper deficiency to be linked with anemia and that iron deficiency is influential on the fetus and the pregnant women's growth during the period of pregnancy [34].

Results of present study in Table 5 agree with that recorded by Najlaa and Iqbal [35], who reported that there was significant difference in Hb value (10.91 g/dL) in second trimester was lower than first trimester group (11.77 g/dL) and the MCV value in second trimester (100 fL) was lower than first (101.6 fL), while the MCH and MCHC values were significantly higher in first trimester than second trimester ($p < 0.05$).

In Thailand Sukrat [36], showed that the value of Hb in pregnant women was (11g/dL) and the value of PCV (Hct) in pregnant was (34.1%), which was lower than non pregnant (37.4%) this reflects that anemia is leading to decrease of the blood Hb and PCV values in pregnant women which lead to increase in plasma blood and decrease in RBC.

Anemia is due to physiological changes in the second trimester of pregnancy period, including the growing needs of iron during pregnancy because of the increase in the blood plasma, which is estimated at about the size of 50% with the expansion in a cell mass of red blood around 25% [32]. In addition to the mitigation (dilution) made in the red blood cells it causes a decline in the level of hemoglobin approximately from 16 weeks and it may increase by 24 weeks of pregnancy period. There is no sufficient stocks of iron [37]. Also, as a result of winning a major expansion in size of blood plasma, it reflects the apparent decrease in the levels of hemoglobin and Hct % [32]. As is clear that the decline in Hb and Hct with low levels of serum ferritin (SF) (less than 15 ng/mL) during pregnancy is an evidence of blood iron deficiency anemia during pregnancy [38].

The results of the statistical test Table 6 in the second trimester of pregnancy presented that the valuables of SI, TIBC, TS and SF were highly significant ($p< 0.01$) in women without anemia and women with anemia. SI average in pregnant women without anemia (13.05±1.305) µmol/L is more than in women with anemia (8.59±0.737). However, TIBC in women without anemia (68.01±3.578) µmol/L is more than in women with anemia (82.01±1.323)

µmol/L. Also, the percentage of TS was (19.11±1.452) % in women without anemia and (10.51±1.323) % in women with anemia. But, SF values were (34.3±1.261) ng/mL in women without anemia and (13.03±1.151) ng/mL in women with anemia.

Table 6: Comparative in indicators of iron (SI, TIBC, TS and SF) for women without anemia and women with anemia in the second trimester of pregnancy.

Parameters	Women without anemia n = 20	Women with anemia n = 20
SI (µmol/L)	13.05±1.305	8.59±0.737 **
TIBC (µmol/L)	3.578±68.01	82.01±1.323 **
TS(%)	19.11±1.452	10.51±1.323 **
SF(ng/mL)	34.3±1.261	13.03±1.151 **

All results are presented as mean ± SD values P<0.01

In the second trimester Table 7 showed that RBC values were (4.183±0.066) vs (3.842±0.162) ×106/mm3 in women without anemia and women with anemia respectively. In contrast RDW in women without anemia was (12.89±0.149) % less than significantly (p<0.01) in women with anemia (15.08±0.505) %. Furthermore, the platelet count was higher in women without anemia (239.95±6.889) ×103/mm3 compared with values of women with anemia (210.25±6.424) ×103/mm3 . But, the MPV in women without anemia was (6.411±0.182) less than in women with anemia (6.302±0.259).

While blood count in second trimester showed in Table 8 , was increased significantly (p< 0.01) from (8.668±0.5) ×109/L in women without anemia to (10.854±0.104) ×109/L in women with anemia Neu, Mono, Eos and Baso increased in women with anemia from women without anemia but Lym in women with anemia decreased than women without anemia.

Table 7: Comparison in blood indicators for all pregnant women in the second trimester of pregnancy (n = 40).

Parameters	Women without anemia n = 20	Women with anemia n = 20
RBC (10^6/mm^3)	4.183±0.066	3.842±0.162 *
RDW(%)	12.89±0.149	15.08±0.505 **
Platelet (10^3/mm$^{3)}$	239.95±6.889	210.25±6.424 *
MPV	6.411±0.182	6.302±0.259

All results are presented as mean ± SD values P<0.01.

Table 8: Comparison in blood indicators among women without anemia and women with anemia in the second trimester of pregnancy .

Parameters	Women without anemia n = 20	Women with anemia n = 20
WBC×10^6/L	8.668±0.500	10.854±0.104**
Neu %	71.75±1.488	74.68±1.878
Lym %	19.87±1.255	17.592±1.417
Mono %	5.385±0.443	6.493±0.533*
Eos %	2.676±0.485	3.578±0.511
Baso %	3.532±0.449	3.9865±0.541

*P< 0.05

Third Trimester of Pregnancy

Results in Table 9 showed the differences between the parameters from pregnant women in third trimester. A highly significant differences (p< 0.01) were found in both of Hb, Hct, MCV, MCH, and MCHC.

Table 9 showed a comparison of blood indicators among women without anemia and women with anemia in third trimester of pregnancy. There was a highly significant (p< 0.01) difference in average of hemoglobin between pregnant women without anemia and

pregnant women with anemia (12.97±0.207) vs (9.65 ±0.327) g/dL respectively. Also, found highly significant (p< 0.01) difference in Hct% reached an average (39.72 ±0.548) vs (30.18 ±0.821)% respectively. Appeared highly significant (p<0.01) in MCV (fL) (86.45 ±0.933) vs (79.08 ±1.691) respectively, also in average of MCH (Pg) (29.82 ±0.438) vs (23.28 ±0.893) respectively and in MCHC (g/dL) average (32.39 ±0.283) vs (28.77 ±0.525) respectively. These results agree with Luay [39].

Table 9: Comparative in indicators in the blood of women without anemia and women with anemia in the third trimester of pregnancy .

Parameters	Women without anemia n = 20	Women with anemia n = 20
Hb (g/dL)	12.97±0.207	9.65±0.327**
Hct (%)	39.72±0.548	30.18±0.821**
MCV (fL)	86.45±0.933	79.08±1.691**
MCH (pg)	29.82±0.438	23.28±0.893**
MCHC (g/dL)	32.39±0.283	28.77±0.525**

All results are presented as mean ± SD values

p<0.01 **

The incidence of anemia in the third trimester of pregnancy is due to the status of iron deficiency in the body under the natural level [40]. In addition, the complication of anemia in the interval pregnancy, includes the weakness in the transfer of oxygen which leads to hypoxia[41].

This could be explained that iron supplements can be reduced to the extent of iron depletion in the third trimester [42]. Blood volume increased by approximately 40 to 45 % above pregnancy levels by the end of the third trimester. Blood plasma and red blood cells (RBCs), both increase in volume, although plasma volume increases at a higher percentage (50%) [43].

The hormonal changes that occur in pregnancy are treatmenous. Rising levels of certain pregnancy hormones cause changes in almost every body system during pregnancy. Changes in the endocrine system itself occur during pregnancy. The pituitary gland in pregnant women larger by 135% than non-pregnant [44]. Most endocrine glands are affected by the increased protein binding that occur during pregnancy. Hyperplasia of glandular tissue and increased vascularity cause the thyroid gland to increase in size the need for insulin is increased [45]. Female sex hormones (estrogen, progesterone, and human gonadotropin) are secreted primarily by the placenta. These hormones are responsible for most physiological changes during pregnancy [45].

Table 10 shows the parameter of iron indicator (SI, TIBC, TS, SF) for the pregnant women in 3rd trimester of pregnancy. All these parameters were highly significant (p< 0.01) differed. SI in women without anemia and women with anemia is (11.71±1.257) vs (7.77±0.705) µmol/L, respectively. TIBC is (79.5±1.323) vs (95.63±2.806) µmol/L, respectively. TS percentage is (14.22±1.225) vs (8.64±1.539) %, respectively. SF is (34.51±1.323) vs (12.19±1.613) ng/mL, respectively.

Table 10: Comparative in indicators of iron (SI, TIBC, TS and SF) for women without anemia and women with anemia in the third trimester of pregnancy.

Parameters	Women without anemia n = 20	Women with anemia n = 20
SI (µmol/L)	11.71±1.257	7.77±0.705 **
TIBC (µmol/L)	79.5±1.323	95.63±2.806 **
TS (%)	14.22±1.225	8.64±1.539 **
SF (ng/mL)	34.51±1.323	12.19±1.613 **

P< 0.01

The third trimester of blood parameters was shown in Table 11, RBC and platelet decreased in women with anemia significantly (p< 0.01) than women without anemia, but the RDW and

MPV in contrast increased in women with anemia than women without anemia.

Table 11: Comparison in blood indicators for all pregnant women in the third trimester of pregnancy (n = 40).

Parameters	Women without anemia n=20	Women with anemia n = 20
RBC (10^6/mm^3)	4.517±0.035	4.097±0.131 **
RDW (%)	13.01±0.161	16.76±0.666 **
Plate (10^9/L)	230.2±8.019	207.05±8.983 **
MPV	6.524±0.322	7.395±0.444

All results are presented as mean ± SD values P<0.01**

Table 12 shows that the WBC counts in third trimester of pregnant women, WBC and neutrophil increased in women without anemia compared with women with anemia, but Lym, Mono, Eos and Baso decreased in women without anemia compared with women with anemia.

Table 12: Comparison in blood indicators among women without anemia and women with anemia in the third trimester of pregnancy .

Parameters	Women without anemia n = 20	Women with anemia n = 20
WBC×10^9/L	12.256±0.975	12.0381±0.959
Neu %	73.24±1.675	72.415±2.051
Lym %	20.12±1.132	20.779±1.308
Mono %	5.274±0.453	5.608±0.437
Eos %	2.1885±0.523	2.502±0.523
Baso %	3.198±0.443	2.778±0.452

*P< 0.05

Mohammed et al showed when evaluation the physiological effect of pregnancy on some hematological and biochemical parameters for pregnant women in Diyala Governorate between (2014-2015) that there significant (p< 0.001) increase in the value of (WBC, neutrophil and monocytes in pregnant women (7.43×109/L, 69.82% and 5.82% respectively) when compared with the mean of control group (6.13×109/L, 59.33% and 4.99% respectively). Also, it shows a significant decrease in the mean of lymphocytes, Hb, PCV and platelets in pregnant women. Furthermore, there was a significant decrease in the mean of RBC in pregnant women 4.41×106/mm3 when compared with the mean of control group 4.97×106/mm3[46]. White blood cells count is used as a clinical marker of innate immune function, the increase in total WBC was due to an increase in the number of circulating neutrophils, granulocytes and monocytes. Alteration in total and differential count of leucocytes may indicate the physiological compensation of the body's defense mechanism through nonspecific immunity, exerted by neutrophils the migratory phagocytes and other leucocytes like monocytes and eosinophil in different trimesters of pregnancy. This alteration in innate immunity represents an attempt to compensate at least partly, the weakened specific immunity of the mother's body [47].

Increase WBC (leukocytosis) which occurs during pregnancy in spite of hemodilution is due to the physiologic stress induced by the pregnant state and because of the increased inflammatory response during normal pregnancy, which can be as a consequence of selective immune tolerance, immunosuppression and immunomodulation of fetus [48]. The stress probably increases hematopoietic activities and blood cell metabolism into circulation [49]. Hemodilution is a well-characterized phenomenon in pregnant women. A decrease in immunoglobulins may be due to hemodilution occurred during pregnancy [50].

Monocytes arise from precursors in the bone marrow and comprise about 5-10% of the circulating blood leukocytes. They have

important function in homeostasis, tissue repair, and inflammation. Not only count of monocytes increase but monocytes are functionally changed in pregnant women [51]. This is, for instance, demonstrated by measuring the production of oxygen free radicals, which is increased in pregnant women. Also monocytes of pregnant women showed increased cytokine production as compared with monocytes from non-pregnant women [51].

Neutrophils are the major type of leucocytes on differential counts. This is likely due to impaired neutrophil apoptosis in pregnancy. The neutrophil cytoplasm shows that toxic granulation, neutrophil chemo taxis and phagocytic activity are depressed, especially due to in hibitory factors present in the serum of a pregnant women [52]. In the advanced stage of gestation there is an endogenous adrenaline release which induces the greater mobilization of neutrophils in the circulation resulting in an increase in total leucocyte count [53]. Leukocyte and neutrophil count increased on day 1 but starts decreasing until fifth day postpartum when the value returns back to normal[48]. This important finding should always be kept in mind to avoid the unnecessary use of antibiotic in the postpartum period.

Decrease in lymphocytes percentage and increase of WBC, neutrophils and monocytes in pregnant women in this study are in agreement with [47][54]. There was also an increase in vital indicators related to innate immunity (total WBC, neutrophils and monocytes) and a decrease in biomarkers related to adaptive immunity (IgA, inspector, immunoglobulin and lymphocytes) during pregnancy.

Nutritional deficiencies, metabolic disorder and changes during pregnancy can be detected by analyzing and monitoring blood and other body fluids [55]. Increases blood volume as early as 6 weeks of pregnancy and increases to more than 50% of the prep regnant state. The change in plasma volume during pregnancy is due to the increased plasma renin activity and lower levels of atrial nitroterric peptides [55]. Changes in RBC, Hb and PCV during pregnancy can be explained as rise in plasma volume more than that compared to increase in the red cell mass, plasma volume increases 25-80% between the 6-24 week of gestation. However, the increase in RBC mass has been found to be 30% between the 12-36 week of gestation when iron and folate are supplemented [56].

A decrease in platelet count during pregnancy is due to hemodilution and partly is due to increased platelet activation and accelerated clearance. It was also suggested to increase platelet consumption as well as decrease the life span in the uterine placenta circulation to be an explanation of the reduction in the number of circulating platelets during pregnancy [48].

White blood cells are responsible for body defense during pregnancy, WBC was reported to be elevated in this study. These results agree with previous work by who asserted that a total lymphocyte count rising in early pregnancy will remain elevated through pregnancy. This happens as a result of the body building of the immunity of the fetus and it is achieved by a state of selective immune tolerance, immunosuppression, and immunomodulation in the presence of a strong antimicrobial immunity. There is also down regulation of potentially dangerous T-cell mediated immune responses, while activating certain components of the innate immune system, such as neutrophils. This unique dysregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy Luppi[57].

On the other side these results agreed with the result of Swapan et al [58].who showed that the leucocyte count was higher throughout pregnancy. This may be a result of the body building of the immunity of the fetus and it is achieved by a state of selective immunotolerance, immunosuppression and immunomodulation in the presence of strong antimicrobial immunity [58]. there was a big difference across all three in the value of WBC

count and packed of cell volume Swapan et al [59]. also showed that the neutrophil%, eosinophil%, monocyte% were 54.15%, 10.51%, 1.8% and 1% respectively in the first trimester, but Swapan in the second trimester these percentage were less than in the first trimester, it was 47.95%, 9.71%, 0.84%, - ,respectively. But the WBC values increased from 6.14 to 7.46 ×109/L in first and second trimester and increased in the third trimester to 8.09×109/L but lymphocyte% also lymphocyte % increased from 33.07 to 40.52% respectively, but in the third trimester, it is decreased to 32.68% . However, WBC is increased in the pregnant women from 7.26 × 109/L companied with control was 4.91× 109/L then lymphocyte % increased in pregnant women from 34.68% to 43.84% in control. Decrease in lymphocytes can be illustrated through that, it has been reported that because it is stimulated by oestrogen, the adrenal cortex produces increasing levels of total and free plasma cortisol and other corticosteroids from 12 weeks to term. It decreased the circulating lymphocyte count, size of lymph node and thymus by inhibiting lymphocyte mitotic activity[47].

Evaluation of red blood cell morphology

Table 13 shows and relies on the instructor of Hb, estimation of prevalence of anemia due to iron deficiency, thalassemia or anemia of chronic disease through different periods of pregnancy. The highest proportion was in the first trimester (90%) in iron deficiency anemia, followed by the second trimester (85%) then third trimester (75%). While the prevalence rate of thalassemia was in the first, second and third trimester 10, 5, 20% respectively. The highest percentage is recorded in the third trimester. The prevalence of anemia of chronic disease (AOCD) was recorded the less percentage 5% in three trimester, it was in first (0%), second (10%) and third trimester (5%).

From these Table 13 the iron deficiency anemia was a greater percentage in 1st , 2nd and 3rd trimester and that was a clear in CBC film. A micrositec is a small cell that has a diameter of less than 7μm and an MCV of less than 80 fL. The hemoglobin content of these cells that lead to weak hemoglobin synthesis may produce micrositec, hypochromic (MCHC < 32% and cells with increased central pallor) blood picture, the result is an increase in cellular divisions and thus a smaller cell in peripheral blood. This form of abnormal hemoglobin synthesis is see in iron deficiency. Deficiency of heme synthesis (sideroblastic anemia), deficiency of globin synthesis (thalassemia) and chronic disease states. Iron deficiency is the most common cause of anemia (IDA) accounting for up to 500 million causes worldwide[60].

Table 13: Prevalence of iron deficiency anemia, thalassemia and anemia of chronic disease (AOCD) in the three pregnancy periods.

Trimester	Iron deficiency Anemia		Thalassemia		AOCD	
	N	%	N	%	N	%
First N=20	18	90	2	10	0	0
Second N=20	17	85	1	5	2	10
Third N=20	15	75	4	20	1	5
Total = 60	50	83.3	7	11.7	3	5

These results agree with other studies, including the results of the study [61].In India for the spread of anemia cases between pregnant women, the incidence of anemia was during pregnancy (in first, second and third trimesters) were 56.6, 70.2 and 69.5% respectively. The highest percentage of anemia was iron deficiency anemia, in third trimester 40.7% compared with second trimester was 35.1%, in Emarat [62], recorded the percentage of iron deficiency anemia was 91%, while the percentage of B-thalassemia anemia was 8%, and the anemia resulting from folate was 1%.

Half of the cases of anemia are caused by iron deficiency and are due to several causes, including under nourishment such as deficiency vitamin A, riboflavin (B2), folic acid, vitamin B12 , in addition to some inflammatory infections such as malaria, and others. A study of Ruwaidah [63], indicated that under hypothyroidism and secondary hypothyroidism were clearly accompanied by

anemia due to iron deficiency, the treatment with levothyroxine hormone for 2-3 months Gokdeniz [64], improves the increase of Hb, MCV, PCV, MCHC and MCH.

There is a link between anemia and other factors like hormone Glutathione (GSH) and Erythropoietin (EPO) hormone. This relationship has been studied by Aaraf and Sahib [65], for pregnant women with gestational anemia in Kirkuk city/ Iraq. This study showed a significant increase ($p< 0.05$) in the level of Erythropoietin hormone during three trimesters of women pregnancy with gestational anemia compared with the three trimesters results of the control groups. Also, it showed a significant decrease in the concentration for Glutathione hormone during the three trimesters compared with the three trimesters to control groups. Erythropoietin (EPO) concentration increased in second and third trimester compared with first trimester, the reason is due to decrease amount of hemoglobin as measured by the amount of blood and thus the lack of oxygen reaching the body organs, including the kidney.

Or the reason may be attributed to physiological changes related to pregnancy and thus increase the need for more oxygen, this means increasing the demand for red blood cells and thus increasing the secretion of the hormone to cover this demand to the formation of more RBC. Or the reason of increase of EPO in second and third trimester may be attributed to a large part of the blood pump which is directed to the uterus and placenta. However, Perewusnyk et al [66], showed an increase in the process of formation of red blood cells thus leads to an increased mass of RBCs. These are preceded by an increased concentration of the hormone. Anemia leads to iron deficiency and an increase in the level of EPO hormone in serum, EPO check produced by kidneys function is targeting growth factors for RBC [67].

Iron requirements markedly increase in the second and third trimester of pregnancy because of high growth rates of fetus and placenta and the development of maternal red cell mass. Therefore, anemia affects 50 to 70% of pregnant women in the developing countries Due to social customs, females get a diet of inferior quality as compared to that of males. Females are more affected, in Pakistan among the selected anemia patients, 72% were iron deficiency anemic and 28% were non-iron deficiency anemic. The risk factors were pregnancy (57%), nutritional inadequacy (36%), Hookworms infection (5%) and others (2%).

Comparison in occupation, monthly income and education between pregnant women with anemia and pregnant women without anemia

Table 14 shows that the highest percentage (90%) of pregnant women with anemia was housewives and about 10% were working women, but less than 83.3% were housewives women, without anemia, and about 16.7% working. The finding of the present study is in agreement with finding, reported in Turkey Nesimikisioglu et al who found that a high proportion of pregnant women (94%) were housewives. This may be a potential cause of health problems during pregnancy. However, these results agreed with the result of Muna who showed that 85.8% of samples in Baghdad were housewives [68].

The level standard of living of the individual and the family plays important role in determine the type of food intake and quantity and thus affect the incidence of anemia. The level of the pension was divided into three categories, which is (low, medium and high) average depending on the mouthy income. The results in Table 14 showed a high incidence of anemia in low living pregnant women (53.3%) followed by medium (30%) and (16.7%) in high monthly income. However, a high percentage of pregnant women without anemia with medium monthly income is (63.3%) followed by low income (26.7%).

Table 14: Distribution of sample according to occupation, monthly income and education .

Variables		Women With Anemia		Women Without Anemia	
		No.	%	No.	%
Occupation	Working	6	10	10	16.7
	House wife	54	90	50	83.3
Monthly income (thousand dinar)	Low	32	53.3	16	26.7
	Medium	18	30	38	63.3
	High	10	16.7	6	10
Education	University and above	12	20	25	48.3
	Junior	19	31.7	21	35
	Elementary School or Less	29	48.3	14	23.3

With the lower standard of living, the concentration of hemoglobin decreased and as a result of the lack of enough food and nutrients necessary for the formation of blood such as meat (rich in iron and protein) and other high priced food. These results agreed with the result of Ismaeil and Hassan who showed that there was positive correlation between the income and families who eat ate quantities of meat prepared in different ways (grilled, fried, and boiled), low income forces pregnant women to eat cheap carbohydrate foods [69]. WHO (1994) showed anemia is common among low income people. But, Sjolin (1981) showed that anemia is spreading in families with a lower socioeconomic level than families with a high socioeconomic level in the United State of America. Also, it agreed with Muthanna et al., [70], since the percentage was found to be 50.4% among low income individuals living in larger families (9 persons). Pregnant women were classified according to educational level to determine the prevalence of anemia as in Table 14 where the prevalence of anemia among pregnant women with low educational level is high (48.3%). On the other hand, pregnant women with a high educational level and above had a prevalence of anemia (20%). On the contrary, in pregnant women without anemia the high percentage (48.3%) in pregnant women have university degree and above, and the less percentage (23.3%) in pregnant women has elementary level or less. It there was a relationship between the level of education and anemia percentage in pregnant women. This is due to the educational and cultural levels of pregnant women which have an impact on their dietary choices and in the preparation of a balanced diet containing all essential nutrients. These results agree with Muthanna et al., Qiaoyi et al, Muna [70] [9] [21].

Conclusions

Increasing level of awareness in women and their families about the risk of anemia in pregnancy and to create the need for iron supplement. The government and ministry of health is aware of the problem of anemia in the country and should implement programs for its control. These programs include fortification of food stuffs with iron, distribution of iron at antenatal and child health clinics, and nutrition education programs. Give iron supplements to all women in reproductive years in urban and rural areas.

Conduct a study on the follow-up to the physiological impact of each of Vitamin B 12 and Folic acid to form the red blood cells process Erythroypoiesis in pregnant women during different periods of pregnancy. And study of the most important hormonal changes in the blood associated with the period of pregnancy in pregnant women during pregnancy, such as different hormone Erythropoietin. Educate pregnant women how dangerous iron deficiency blood anemia during pregnancy and what caused the miscarriages and other risks Health and encourage pregnant mothers about important family planning and good nutrition. Conduct a study on measuring RBC Ferritin, protoprophyrin , Transferrin receptors in pregnant women during different periods of pregnancy.

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