**Abstract**—The mother’s body undergoes several changes in all organ of the bodily systems to stimulate the growth and development of the fetus; hence, the clinical laboratory play an important role in the management of pregnant women. A total number of 90 sample were (60 pregnant and 30 control) from the general women population that were referrals from al-Ramadi of obstetrician – gynecologist hospital in the period November 2021 to June 2022. The result of statistical analysis of the present study showed there were no significantly differences between age of pregnant and control group and the gestation period time the pregnant women was 15 % in 1st trimester of gestation, 35 % in 2nd trimester of gestation and 50 % in 3rd trimester of gestation , in addition complete peripheral blood count examination ( leukocytes , Neutrophils , Lymphocytes ,Erythrocytes , Hemoglobin , Hematocrit , and Platelet) revealed significantly lower rates of pregnancy compared to the control group while (Neutrophil) were revealed significantly increase between pregnant and control group. The biochemical alterations (Iron, Ferritin, Hepcidin, B12 and Homocystiene) were revealed significantly decrease between pregnant
and control group while (Total iron binding capacity) were revealed significantly increase between pregnant and control group. In conclusion normal pregnancy is associated with many physiological changes which includes Leukocytosis, Neutrocytosis, Lymphocytopenia, anemia, and thrombocytopenia in addition the iron status have the important role in diagnosis of anemia in pregnant women as compared with control group.

**Keywords**--hepcidin, B12, homocystiene, total iron binding capacity.

**Introduction**

Pregnancy is development of one or more embryo in a uterus of the woman's after the fertilization process (1). The state of pregnancy is characterized by occurrence of numerous physiologic changes that affect in almost every organ and all system in the body and these changes are essential to maintain a successful pregnancy and prepare the body for delivery and lactation as well as to satisfy the requirements of the fetoplacental unit (2 and 3). The relationship between pregnancy and the hematological parameters are includes the changes in leukocytes, erythrocytes, hemoglobin concentration, hematocrit, and thrombocytes (4,5) The alterations of these parameters are known as “physiologic anemia of pregnancy” (6). Anemia has been the most prevalent nutritional deficiency affecting pregnant women of both genders and all ages (7) It is believed that the biological changes of physiologic anemia reduce blood viscosity in facilitating the vascular permeability and perfusion of the newly developed uteroplacental components. As a result, the developing baby receives adequate oxygen and nutrients (8).

During pregnancy, Adequate maternal nutrition is required to support fetal growth and development (9). The master regulator a hormone of iron absorption and reuse that produced by the liver is known as Hepcidin. Hepcidin is upregulated by inflammation and iron content and reduce by erythropoietic demand and hypoxia (10). A woman's nutritional status during pregnancy is not only critical for her health, but also for that of future offspring (11). Vitamin D deficiency is worldwide epidemic problem and a prevalent metabolic disorder, and it plays a crucial role in the body's growth and development. As a result, it is essential to supply vitamin deficiencies during pregnancy in order to reduce the risk of undesirable perinatal consequences. During pregnancy, there is a physiological hemodilution that leads to decrease in the plasma levels of some vitamins, whereas the plasma levels of other vitamins are not changed because carrier proteins are increased (12 -14). Vitamin B12 helps to maintain healthy folate metabolism, which is essential for the cell proliferation gestation period. Although there is no conclusive evidence of vitamin B12 deficiency, it is commonly reported as a result of insufficient nutrition food intake and a physiological fall in the level of maternal vitamin B12. Increased maternal metabolism and active placental transport explain this decrease. If the pregnant women have deficient in vitamin B12 during gestation, the newborn may have low vitamin B12 levels (15).
Subject and Methods

The present study was conducted sixty pregnant women during all period of trimester and thirty non pregnant women as control. al-Ramadi of obstetrician – gynecologist hospital in the period November 2021 to June 2022. The socio-demographic information including (age and sex) and clinical data were collected. Additionally, Blood collected drawing by venipuncture and put into to two tubes, one is a sterile blood collection tube with dipotassium ethylene diamine tetra-acetic acid vacutainer to laboratory investigations for performed complete blood count analysis by using an automated procedure (Sysmex 2000). Another tubes is gel tubes to collect the serum when the Blood collected drawing put on t and left to coagulate at temperature of the room for about an hour. Then collected the serum after centrifugation at 1000 rpm for 30 minutes. The serum was kept in the deep freezer (-20°C) until it was used to measure hepcidin using a sandwich ELISA kit from Sincere Biotech in China and (S. Iron, Total Iron Binding Capacity were estimated from all the samples using the Ferrozine method and a colorimetric kit provided by Coral clinical. Goa, India 403202. S.Iron, Ferritin, Vitamin D, Vitamin B12, and homocystein were tested using an electrochemiluminescence immunoassay on Cobas c311 analyzer (Roche, Germany).

Statistical analysis

Data obtained were subjected to fund as mean ± standard deviation (SD) and considered statistically significant when the p value ≤0.05. Statistical analysis done using the statistical analyzing system (Graph pad prism). For comparisons the analyses between pregnant women non pregnant women (control group).

Result

Age and Trimester period distribution of pregnant women

The study was carried out in women attending Al-ramadi teaching department of gynecology and obstetrics. The mean value of age for pregnant were (27.1 ± 6.290) while the mean value of age for control was (27.0 ± 5.762) therefore there were no significantly differences in pregnents and control. The gestation period time the pregnant women was divided into three groups: 15 % in 1st trimester of gestation, 35 % in 2nd trimester of gestation and 50 % in 3rd trimester of gestation (Table 1).

| Trimester       | Number | Percentage (%) |
|-----------------|--------|----------------|
| First trimester | 9      | 15             |
| Second trimester| 21     | 35             |
| Third trimester | 30     | 50             |
| Total           | 60     | 100            |

Table 1
The distribution of pregnant women by trimester state
Hematological parameters of the study population

Complete peripheral blood count examination in the current study (Leukocytes, Lymphocytes, Erythrocytes, Hemoglobin, Hematocrit and Thrombocytes) revealed significantly decrease in pregnant in compared with control group while (Neutrophil) were revealed significantly increase in pregnant in compared with control group. These results were showed in table (2).

| Hematological parameters | Non pregnant (control) No.(30) | Pregnants No.(60) | P value |
|--------------------------|-------------------------------|-----------------|--------|
| Leukocytes (10^3/mm^3)   | 8.056±1.463                  | 12.07±1.7       | <0.0001* |
| Neutrophil (10^3/mm^3)   | 3.994±1.118                  | 9.119±2.545     | <0.001*  |
| Lymphocytes (10^3/mm^3)  | 1.882±0.4716                 | 3.193±1.834     | 0.0033* |
| Erythrocytes (10^3/mm^3) | 4.327±0.5769                 | 3.072±0.6584    | <0.0001* |
| Hemoglobin               | 12.32±1.45 (g/dl)            | 7.723 ±1.278 (g/dl) | <0.0001* |
| Hematocrit(%)            | 36.46±4.22                   | 24.57±4.215     | <0.0001* |
| Thrombocytes (10^3/mm^3) | 253.7±54.25                  | 127.8±17.15     | <0.0001* |

Biochemical alterations of the study population

The biochemical alterations of the current study included (Iron, Ferritin, Hepcidin, B12 and Homocystiene) were revealed significantly decrease between pregnant and compared with control group while (Total iron binding capacity) were revealed significantly increase between pregnant and compared with control and these results were showed in table (3).

| Biochemical alterations     | Non pregnant (control) No.(30) | Pregnant No.(60) | P value |
|-----------------------------|---------------------------------|-----------------|--------|
| Iron                        | 88.86 ±36.37 µg/dl              | 27.4 ±4.53 µg/dl | <0.0001* |
| Hepcidin                    | 13.86 ±7.905 ng/ml              | 0.8123 ± 0.208 ng/ml | <0.0001* |
| Vitamin D3                  | 37.75±4.678 ( ng/ml)            | 10.3±2.806 ( ng/ml) | <0.0001* |
| Ferritin                    | 45.25 ± 24.83 ng/ml             | 7.3 ± 2.054 ng/ml | <0.0001* |
| Total iron binding capacity | 405.2 ±26.48 µg/dl              | 587.6 ±73.93 µg/dl | <0.0001* |
| B12                         | 449.8 ± 140.2 pg/ml             | 126.6 ±27.6 pg/ml | <0.0001* |
| Homocystiene                | 8.245 ±1.057 µmol/L             | 2.05 ±0.4539 µmol/L | <0.0001* |

Discussion

During pregnancy, a woman undergoes several physiological changes in all body system. As a result of these changes (16) Increases in the mean number of white
blood cells and Neutrophil in pregnant revealed significant differences in compared with control group. According to the findings of Pughikumo et al., these values changed as the gestational age advanced. According to the findings of Pughikumo et al., these values changed as the gestational age progression and the present study agrees with previous work by Luppi studies who reported Leukocytosis, occurrence during pregnancy is a result of the physiological stress caused by the pregnant state. (17 and 18). This leukocytosis results in gestation state and the increase in Neutrophils are probably homeostatic response to an apoptosis of the altered expression of neutrophils during gestation. (19). In addition the an increase of leukocytes is mainly associated with to an increase of the number of Neutrophils (20).

There is some evidence that neutrophils have an enhanced rate of oxidative metabolism during pregnancy. The chemotaxis and phagocytic activity of neutrophils are significantly reduced, particularly as a result of inhibitory substances that are found in the serum of a pregnant woman (21). Lymphocyte count were significantly lower through the 1st and 2nd trimester of gestation and little increases during the 3rd trimester of gestation (22). These result of lymphocytopenia is in agreement with Gökçen Örgül et al who noted the reduce in lymphocyte count is mainly due to gestational and accompanying hormonal alterations, which have a detrimental influence on the total blood lymphocyte count (23).

In the present study the count of the platelet for pregnant was significantly lowerin compared with control group. This thrombocytopenia is accompanied by platelet hyperreactivity to aggregating agents, which is associated with an increased production of thromboxane A2. It is caused in part by hemodilution, increased platelet activation, and accelerated clearance (24) additionally, thrombocytopenia as gestation progresses, there is evidence that the width of the platelet volume distribution rises significantly and constantly for the reasons already stated. Consequently, as gestation advances, the mean thrombocyte volume becomes an insensitive indicator of thrombocyte size (25).

In current study we showed the physiological changes to some of the basic hematological parameters during all trimesters of pregnancy such as Erythrocytes, Hemoglobin and Hematocrit were have the clinically lower significant differences between the pregnant and compared to control group. All cells require iron for oxygen supply, electron transport, and enzyme function. High-metabolic-rate cells demand more iron and are more sensitive to malfunction in the presence of an iron deficiency. As the fetus grows and the mother's blood volume increases, the fetus receives more oxygen. Pregnancy is therefore a situation of impending or present iron insufficiency, which can be difficult to diagnose (26). In the context of what is commonly referred to as the physiologic anemia of pregnancy, iron deficiency has an influence on red cell formation. This characteristic is shared among all mammalian species (27). It is theorized that the natural anemia that occurs during pregnancy improves placental perfusion by decreasing the viscosity of maternal blood and enabling the transfer of oxygen and nutrients to the fetus by increasing the erythrocyte mass (28).
Because of the rebalance between volume of the plasma and red blood cells mass, it is commonly believed that a Hemoglobin levels of 11 g/dL in the late 1st trimesters and 10 g/dL in the 2nd and 3rd trimesters of gestation should be investigated for a cause other than the physiologic anemia of pregnancy (29). These findings are similar to Geetanjali et al. who reported the gradual decrease in Hemoglobin levels from the 1st to the 3rd trimesters of pregnancy which be the result of an increased of iron need due the gestation period advances. To accommodate the increase in maternal Hb mass and fetal growth needs, more iron is necessary. The increased estrogen and progesterone that the placenta secretes during gestation lead the kidneys to release renin. Renin promotes the aldosterone - renin-angiotensin pathway, causing the retention of sodium and expansion the volume of plasma. The 25–80 percent rise in plasma volume between the 6 and 24 week of gestation is significantly higher than the growth in red blood cells mass, resulting in a decrease in maternal Hemoglobin and physiological anemia (30 and 31).

The levels of hemoglobin has been used as an indicator for iron deficiency. This provides a quantitative estimate the iron deficiency severity after anemia has evolved, but it lacks sensitivity and specificity and should not be the sole indicator of iron status (32). For practical clinical application, other authors, in some previous studies run in industrialized nations, suggested a rate of hematocrit lower than 30 % as the discriminatory value of anemia for pregnant woman (33).lower Hematocrit level is due to the hemodilution caused by the increase the volume of blood and elevated the rate of glomerular filtration, and the fetus could absorb a proportion of hematocrit during gestational period (34). In previous research conducted in industrialized nations, other authors suggested a rate of hematocrit less than 30 percent as the discriminating criterion for anemia in pregnant women (33). During pregnancy, the fetus may absorb a portion of the mother's Hematocrit due to the hemodilution generated by an increase in blood volume and glomerular filtration rate (34).

This study suggests that hepcidin, hemoglobin, serum iron, serum ferritin and Total iron binding capacity is essential indicator of anemia in pregnant women(35). Pregnancy is a physiological condition that often does not influence a pregnant woman's general health. Nevertheless, pregnancy causes hormonal, hemodynamic, hematological, and certain biological alterations (36 and 37). Iron is required during gestation for: 1) the growth of the placenta and develop of fetus; 2) the increase in the maternal erythrocytes mass; 3) the replenishment of blood losses upon parturition; and 4) the restoration of iron losses (38). The present study, pregnant women had iron deficiency at any stage. The prevalence of iron deficiency was extremely high, especially considering that this illness is preventable through dietary guidance and the use of iron supplements during pregnancy (39). Women with iron stores have a smaller increase than those with iron deficiency. This inability to expand plasma volume may disguise a decrease in hemoglobin content in some iron-deficient women (40).

Understanding the physiological processes of hepcidin has allowed the pathogenic mechanisms of anemia to be identified.Various types of anemia are clinical symptoms of both iron deficiency and excess, given that iron is an essential component of hemoglobin. This seeming contradiction may be explained
by the presence of hepcidin. Hepcidin is the principal iron metabolism regulator in the human body. Hepcidin decreases the quantity of iron in circulation by promoting ferroportin breakdown, which leads to tissue iron sequestration and decreased intestine absorption. In different physiological conditions, such as pregnancy, anemia of chronic disease, myelodysplastic syndromes, and -thalassemia, altered hepcidin concentration is a compensatory strategy to restore iron homeostasis (41).

In healthy pregnancies, maternal hepcidin concentrations are gradually reduced during the 2nd and 3rd trimesters, and they are almost undetectable by the end of the gestational period. (42). In iron deficiency, hepcidin concentrations are reduced, allowing for higher iron absorption and utilization, as well as elevated iron loading and inflammation, which prevent iron access to the plasma. Therefore, there is tremendous interest in developing hepcidin as an iron status diagnostic test (43 and 44). During the gestational stages, a decrease in circulation hepcidin levels suggests pregnant iron deficiency it is essential for the preservation of healthy maternal and homeostasis of embryonic iron (45). Additionally, a reduction in serum hepcidin levels Improves maternal iron absorption and placental transfer of dietary ironresulting in an improvement in the newborn iron status (46). It is yet unknown how hepcidin levels are regulated throughout pregnancy. Outside of pregnancy, hepcidin is downregulated by low iron stores, hypoxia, and anemia (such as erythropoietic activity), while it is raised by inflammation or high iron stores (47 -49).

In contrast, elevated levels of hepcidin reduce dietary iron absorption and impair the efficiency of pregnant iron supplementation and the transfer of maternal iron to the fetus, resulting in decreased iron bioavailability in the fetus. Low maternal hepcidin levels may allow the iron to enter the circulation – placenta - neonate axis and facilitate iron endowment to the fetus (50). The assumption is that a comparatively low concentration of hepcidin in late-pregnant women limits iron buildup. The comparatively high iron content of the stored reserves may account for the higher hepcidin concentrations in full-term infants compared to their mothers. Neonatal iron status was independent of maternal or cord blood hepcidin levels (51). A balanced diet covers the greater nutrient requirements with the exception for vitamin D, and iron(52). Vitamin D deficiency in pregnancy may affect women as well as their newborn (53). Around the world, both vitamin-D and Iron (Fe) deficiencies are considered to be global common problem during the pregnant women and have been significantly associated with a number of adverse pregnancy outcomes such as elevated risk for Gestational diabetes mellitus, anemia, and preeclampsia (54 and 55).

The concentration of ferritin in the bloodstream is an obvious indicator of iron reserves. It is the most specific sign of depleted iron storage, especially when used in conjunction with other iron status tests. In light of the above information, it is evident that estimations of hemoglobin, iron, ferritin, TIBC, and Fe/TIBC percent are useful criteria for determining iron insufficiency in pregnancy. Using various combinations of measurements to improve the specificity of prevalence estimates or to define the various stages of iron deficiency is a more varying stages efficient strategy (56). Several criteria have been used to diagnose iron deficiency anemia. Hemoglobin levels, iron, and bone marrow examination. Faults, however, have
been found with each of these methods. Recently, depressed iron saturation of transferrin (Fe/TIBC) is a reliable method of diagnosing iron deficiency anemia (57).

The total iron-binding capacity gradually increased throughout pregnancy (58). Significantly low blood iron and elevated TIBC in pregnant women are partly due to iron deficiency in the diet. Therefore, iron therapy during pregnancy is beneficial for maintaining serum iron and TIBC levels nearer to those of average non pregnant women (59). The increase in iron-binding capacity during the 2nd second trimester of gestational period occurs when the fetus has the greatest iron requirement. There is a close relationship between the decrease in serum iron and the rise in iron binding capacity, as shown by the present observations. If these changes were due to variations in plasma volume, the deviations would be in the same direction (60). As iron stores deplete, the amount of transferrin which is available to bind iron increases. TIBC significantly increased with gestational age, as expected, during pregnancy (61).

deficiency of Micronutrient persists throughout the world, and although it is greater in low-resource settings, 'hidden hunger' is also frequent in wealthy nations. Due to their high vitamin and mineral requirements compared to their energy intake, young women and children are especially vulnerable to hidden hunger. We consider how iron, iodine, and vitamin D insufficiency might dramatically impair prenatal health and newborn development, given that they share several risk factors and have overlapping effects (62). In all morphological types of anemia, all deficiencies of nutrient (Ferritin, Vitamin D, and Vitamin B12) were detected. 73.26 percentage of iron-deficient anemic women is significantly and also lacked folate or vitamin B12, indicating that further strategies are required to reduce the prevalence of anemia. Two-thirds of the women in our study were vegetarians, which contributed to the high incidence of women with vitamin B12 deficiency (63).

Deficiency of Vitamin B₁₂ has been linked with an increased risk of gestational complications and adverse birth outcomes such as preeclampsia, spontaneous abortion, intrauterine growth restriction, preterm labor, neonatal megaloblastic anemia, neural tube abnormalities, and neonatal neurological problems. The present study explored the association of vitamin B12 deficiency and marginal vitamin B12 deficiency with various socio-economic, and diet-related factors (64-66). Low maternal vitamin B12 levels are closely related to lower vitamin B12 levels in cord blood. vitamin B12 and Folate are necessary cofactors for metabolism of homocysteine (67). Homocysteine is marker disorder in one-carbon metabolism. Homocysteine levels may serve as a link between inadequate maternal nutrition and impaired embryonic and fetal development (68). In normal pregnancies, plasma homocysteine levels decrease physiologically. This is thought to be a physiological adaptation (69). Normal maternal homocysteine levels decrease with increasing gestational age. It may be a normal pregnancy reaction or the result of decreased albumin, increased estrogen, hemodilution caused by plasma volume increase, and an increase in the demand for methionine by the mother and fetus. Fetal utilization is another hypothesized method. Significant correlations exist between maternal vitamin B12, folate, homocysteine levels, and cord blood levels (70).
Conclusion

In conclusion the pregnancy period have significant hematological changes which includes physiological anemia, Leukocytosis, Neutrocytosis, Lymphocytopenia, thrombocytopenia and the iron status have the important role in diagnosis of anemia in pregnant women as compared with control group. Analyze utilization for anemia screening during pregnancy can be improved to guide patient management to reduce anemia potential anemia-related complications.

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Conflicts of interest

The authors declare no conflict of interest.

Authors Contributions

Conceptualization, H.M.S and T.M.M; methodology, H.R.A.A; validation, D.A.S. and H.R.A.A.; formal analysis, T.M.M. and; data curation, H.R.A.A and H.R.A.A; writing—original draft preparation, H.M.S; writing—review and editing, T.M.M., H.R.A.A; supervision, D.A.S.; funding acquisition, M†††, All authors have read and agreed to the published version of the manuscript.

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