How to Cite:
Saleem, H. M., Muhammed, T. M., Al-Hetty, H. R. A. K., & Salman, D. A. (2022). Physiological, hematological and some biochemical alterations during pregnancy. *International Journal of Health Sciences*, *6*(S6), 7156–7169. https://doi.org/10.53730/ijhs.v6nS6.12017

Physiological, hematological and some biochemical alterations during pregnancy

Hiba Muwafaq Saleem
Department of Medical Laboratory Techniques, Al-Maarif University college, AL-Anbar, Iraq
Email: h.m.saleem@uoa.edu.iq

Thikra Majid Muhammed
Department of Medical Laboratory Techniques, Al-Maarif University college, AL-Anbar, Iraq
Email: th.m.mohamed@uoa.edu.iq

Hussein Riyadh Abdul Kareem Al-Hetty
Department of Nursing, Al-Maarif University college, AL-Anbar, Iraq
Email: hussain.riyad@uoa.edu.iq

Dina Akeel Salman
Department of Obstetrics and Gynecology, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq
Email: Dina.akeel@uomustansiryah.edu.iq

***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 (-20oC) until it was and 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 pregnants

The study was carried out in women attending Al-ramadi teaching department of gynecology and obstetrics. The mean value of age for pregnants 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 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).

Table 1
The distribution of pregnant women by trimester state

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

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).

Table 2
Hematological parameters of the study subjects (mean ± SD)

Hematological parameters	Non pregnants (control) No.(30)	Pregnants No.(60)	P value
Lukocytes ($10^3/mm^3$)	8.056±1.463	12.07±1.7	<0.0001*
Neutrophil ($10^3/mm^3$)	3.994±1.118	9.119±2.545	<0.0001*
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).

Table 3
Biochemical alterations of the study subjects (mean ± SD)

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 peroid. (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 pregnent 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 itis 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 B_{12} 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.

Acknowledgment

Special thanks to Al-Maarif University college for allowing us to utilize their laboratories. We would also want to thank all participants for their collaborations and agreement to fill out the questionnaire in order to complete of the research

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.

References

1. Abbassi-Ghanavati, M., Greer, L. G., & Cunningham, F. G. (2009). Pregnancy and laboratory studies: a reference table for clinicians. *Obstetrics & Gynecology*, *114*(6), 1326-1331.
2. Akinbami, A. A., Ajibola, S. O., Rabiu, K. A., Adewunmi, A. A., Dosunmu, A. O., Adediran, A., ... & Ismail, K. A. (2013). Hematological profile of normal pregnant women in Lagos, Nigeria. *International journal of women's health*, *5*, 227.
3. Amah-Tariah, F. S., Ojeka, S. O., & Dapper, D. V. (2011). Haematological values in pregnant women in Port Harcourt, Nigeria II: Serum iron and transferrin, total and unsaturated iron binding capacity and some red cell and platelet indices. *Niger J Physiol Sci*, *26*(2), 173-8.
4. Annuradha, V. K. T., & Suganya, S. Correlation Between Plasma Homocysteine Concentration and Early Onset Severe Preeclampsia. *Renal failure*, *11*(11), 6-10805.
5. Bah, A., Pasricha, S. R., Jallow, M. W., Sise, E. A., Wegmuller, R., Armitage, A. E., ... & Prentice, A. M. (2017). Serum hepcidin concentrations decline during pregnancy and may identify iron deficiency: analysis of a longitudinal pregnancy cohort in the Gambia. *The Journal of nutrition*, *147*(6), 1131-1137.
6. Bakrim, S., Motiaa, Y., Ouarour, A., & Masrar, A. (2018). Hematological parameters of the blood count in a healthy population of pregnant women in

the Northwest of Morocco (Tetouan-M'diq-Fnideq provinces). *Pan African Medical Journal*, *29*(1), 1-12.
7. Bakrim, S., Motiaa, Y., Ouarour, A., & Masrar, A. (2018). Hematological parameters of the blood count in a healthy population of pregnant women in the Northwest of Morocco (Tetouan-M'diq-Fnideq provinces). *Pan African Medical Journal*, *29*(1), 1-12.
8. Bakrim, S., Motiaa, Y., Ouarour, A., & Masrar, A. (2018). Hematological parameters of the blood count in a healthy population of pregnant women in the Northwest of Morocco (Tetouan-M'diq-Fnideq provinces). *Pan African Medical Journal*, *29*(1), 1-12.
9. Bener, A., Al-Hamaq, A. O., & Saleh, N. M. (2013). Association between vitamin D insufficiency and adverse pregnancy outcome: global comparisons. *International journal of women's health*, *5*, 523.
10. Bonnar, J., & Goldberg, A. (1969). The assessment of iron deficiency in pregnancy. *Scottish Medical Journal*, *14*(6), 209-214.
11. Cikot, R. J., Steegers-Theunissen, R. P., Thomas, C. M., de Boo, T. M., Merkus, H. M., & Steegers, E. A. (2001). Longitudinal vitamin and homocysteine levels in normal pregnancy. *British Journal of Nutrition*, *85*(1), 49-58.
12. El-Amin, R., Custer, L., & Silk, J. (2022). Normal physiology of pregnancy. In *Endocrine Diseases in Pregnancy and the Postpartum Period* (pp. 2-6). CRC Press.
13. Erkkola, M., Karppinen, M., Järvinen, A., Knip, M., & Virtanen, S. M. (1998). Folate, vitamin D, and iron intakes are low among pregnant Finnish women. *European journal of clinical nutrition*, *52*(10), 742-748.
14. Fay, J., Cartwright, G. E., & Wintrobe, M. M. (1949). Studies on free erythrocyte protoporphyrin, serum iron, serum iron-binding capacity and plasma copper during normal pregnancy. *The Journal of Clinical Investigation*, *28*(3), 487-491.
15. Finkelstein, J. L., Guillet, R., Pressman, E. K., Fothergill, A., Guetterman, H. M., Kent, T. R., & O'Brien, K. O. (2019). Vitamin B12 status in pregnant adolescents and their infants. *Nutrients*, *11*(2), 397.
16. Fisher, A. L., & Nemeth, E. (2017). Iron homeostasis during pregnancy. *The American journal of clinical nutrition*, *106*(suppl_6), 1567S-1574S.
17. Fisher, A. L., & Nemeth, E. (2017). Iron homeostasis during pregnancy. *The American journal of clinical nutrition*, *106*(suppl_6), 1567S-1574S.
18. Gandamayu, I. B. M., Antari, N. W. S., & Strisanti, I. A. S. (2022). The level of community compliance in implementing health protocols to prevent the spread of COVID-19. International Journal of Health & Medical Sciences, 5(2), 177-182. https://doi.org/10.21744/ijhms.v5n2.1897
19. Ganz, T., & Nemeth, E. (2012). Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1823*(9), 1434-1443.
20. Georgieff, M. K. (2020). Iron deficiency in pregnancy. *American journal of obstetrics and gynecology*. mmonly used biomarkers such as hemoglobin and ferritin concentrations.
21. Girelli, D., Nemeth, E., & Swinkels, D. W. (2016). Hepcidin in the diagnosis of iron disorders. *Blood, The Journal of the American Society of Hematology*, *127*(23), 2809-2813.
22. Hansen, R., Spangmose, A. L., Sommer, V. M., Holm, C., Jørgensen, F. S., Krebs, L., & Pinborg, A. (2022). Maternal first trimester iron status and its

association with obstetric and perinatal outcomes. *Archives of Gynecology and Obstetrics*, 1-13.
23. Harrison, K. A. (1966). Blood volume changes in normal pregnant Nigerian women. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, *73*, 717-723.
24. Hytten, F. (1985). Blood volume changes in normal pregnancy. *Clinics in haematology*, *14*(3), 601-612.
25. Jouanne, M., Oddoux, S., Noël, A., & Voisin-Chiret, A. S. (2021). Nutrient requirements during pregnancy and lactation. *Nutrients*, *13*(2), 692.
26. Kaushal, S., Priya, T., Thakur, S., Marwaha, P., & Kaur, H. (2022). The Etiology of Anemia Among Pregnant Women in the Hill State of Himachal Pradesh in North India: A Cross-Sectional Study. *Cureus*, *14*(1).
27. Kiely, M. E., McCarthy, E. K., & Hennessy, Á. (2021). Iron, iodine and vitamin D deficiencies during pregnancy: epidemiology, risk factors and developmental impacts. *Proceedings of the Nutrition Society*, *80*(3), 290-302.
28. Koenig, M. D., Tussing-Humphreys, L., Day, J., Cadwell, B., & Nemeth, E. (2014). Hepcidin and iron homeostasis during pregnancy. *Nutrients*, *6*(8), 3062-3083.
29. Koenig, M. D., Tussing-Humphreys, L., Day, J., Cadwell, B., & Nemeth, E. (2014). Hepcidin and iron homeostasis during pregnancy. *Nutrients*, *6*(8), 3062-3083.
30. Koller, O. (1982). The clinical significance of hemodilution during pregnancy. *Obstetrical & gynecological survey*, *37*(11), 649-652.
31. Konijnenberg, A., Stokkers, E. W., van der Post, J. A., Schaapb, M. C., Boer, K., Bleker, O. P., & Sturk, A. (1997). Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell adhesion molecules. *American journal of obstetrics and gynecology*, *176*(2), 461-469.
32. Kulik-Rechberger, B., Kościesza, A., Szponar, E., & Domosud, J. (2016). Hepcidin and iron status in pregnant women and full-term newborns in first days of life. *Ginekologia Polska*, *87*(4), 288-292.
33. Letsky, E. (2004). Haematology of pregnancy. *Medicine*, *32*(5), 42-45.
34. Lund, C. J., & Donovan, J. C. (1967). Blood volume during pregnancy: significance of plasma and red cell volumes. *American journal of obstetrics and gynecology*, *98*(3), 393-403.
35. Luppi, P. (2003). How immune mechanisms are affected by pregnancy. *Vaccine*, *21*(24), 3352-3357.
36. Lynch, S., Pfeiffer, C. M., Georgieff, M. K., Brittenham, G., Fairweather-Tait, S., Hurrell, R. F., ... & Raiten, D. J. (2018). Biomarkers of Nutrition for Development (BOND)—iron review. *The Journal of nutrition*, *148*(suppl_1), 1001S-1067S.
37. Manios, Y., Moschonis, G., Lambrinou, C. P., Tsoutsoulopoulou, K., Binou, P., Karachaliou, A., ... & Cashman, K. D. (2018). A systematic review of vitamin D status in southern European countries. *European journal of nutrition*, *57*(6), 2001-2036.
38. Milman, N., BYG, K. E., & Agger, A. O. (2000). Hemoglobin and erythrocyte indices during normal pregnancy and postpartum in 206 women with and without iron supplementation. *Acta Obstetricia et Gynecologica Scandinavica: ORIGINAL ARTICLE*, *79*(2), 89-98.

39. Muhammed, T. M., Saleem, H. M., & Almawla, S. O. (2018). Evaluation of Seminal Plasma Anti-Mullerian Hormone Levels and Their Association with Sperms' Count and Activity in Infertile Males. *Indian Journal of Public Health Research & Development*, *9*(11).
40. Örgül, G., Soyak, B., Portakal, O., Beksaç, M., & Beksaç, M. S. (2017). Total blood lymphocyte count alteration during and after pregnancy. *Gynecology Obstetrics & Reproductive Medicine*, *23*(1), 11-13.
41. Osonuga, I. O., Osonuga, O. A., Onadeko, A. A., Osonuga, A., & Osonuga, A. A. (2011). Hematological profile of pregnant women in southwest of Nigeria. *Asian Pacific Journal of Tropical Disease*, *1*(3), 232-234.
42. Paiva, A. D. A., Rondó, P. H., Pagliusi, R. A., Latorre, M. D. R., Cardoso, M. A., & Gondim, S. S. (2007). Relationship between the iron status of pregnant women and their newborns. *Revista de Saúde Pública*, *41*, 321-327.
43. Pascual, Z. N., & Langaker, M. D. (2021). Physiology, pregnancy. In *StatPearls [Internet]*. StatPearls Publishing .
44. Pobee, R. A., Setorglo, J., Klevor, M., & Murray-Kolb, L. E. (2021). The prevalence of anemia and iron deficiency among pregnant Ghanaian women, a longitudinal study. *PloS one*, *16*(3), e0248754.
45. Pughikumo, O. C., Pughikumo, D. T., & Omunakwe, H. E. (2015). White blood cell counts in pregnant women in Port Harcourt, Nigeria. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, *14*(3), 01-03.
46. Purohit, G., Shah, T., & Harsoda, J. M. (2015). Hematological profile of normal pregnant women in Western India.
47. Ramsay, M. (2010). Normal hematological changes during pregnancy and the puerperium. *The obstetric hematology manual*, 1-11.
48. Rauf, A., Shariati, M. A., Khalil, A. A., Bawazeer, S., Heydari, M., Plygun, S., ... & Aljohani, A. S. (2020). Hepcidin, an overview of biochemical and clinical properties. *Steroids*, *160*, 108661.
49. Rauf, A., Shariati, M. A., Khalil, A. A., Bawazeer, S., Heydari, M., Plygun, S., ... & Aljohani, A. S. (2020). Hepcidin, an overview of biochemical and clinical properties. *Steroids*, *160*, 108661.
50. Raza, N., Sarwar, I., Munazza, B., Ayub, M., & Suleman, M. (2011). Assessment of iron deficiency in pregnant women by determining iron status. *Journal of Ayub Medical College Abbottabad*, *23*(2), 36-40.
51. Robeck, T.R.; Nollens, H.H. Hematological and serum biochemical analytes reflect physiological challenges during gestation and lactation in killer whales (Orcinus orca). Zoo Biol. 2013, 32, 497–509.
52. Roberts, W. E., & Morrison, J. C. (1987). Evaluation of anemia in pregnancy. *Hematologic Problems in Pregnancy. Oradell, Medical Economics Books*, 15-26.
53. Rubini, E., Snoek, K. M., Schoenmakers, S., Willemsen, S. P., Sinclair, K. D., Rousian, M., & Steegers-Theunissen, R. P. (2022). First Trimester Maternal Homocysteine and Embryonic and Fetal Growth: The Rotterdam Periconception Cohort. *Nutrients*, *14*(6), 1129.
54. Saleem, H. M., Muhammed, T. M., & Almawla, S. O. G. (2021). The Association between Overweight and Some Biological Markers among Fewer than 6 Years Aged Children. *Annals of the Romanian Society for Cell Biology*, *25*(6), 10138-10146.

55. Sangkhae, V., Fisher, A. L., Chua, K. J., Ruchala, P., Ganz, T., & Nemeth, E. (2020). Maternal hepcidin determines embryo iron homeostasis in mice. *Blood*, *136*(19), 2206-2216.
56. Sangkhae, V., Ganz, T., & Nemeth, E. (2020). Maternal Hepcidin Suppression Is Essential for Healthy Pregnancy. *Blood*, *136*, 43-44.
57. Shi, H., Chen, L., Wang, Y., Sun, M., Guo, Y., Ma, S., ... & Qiao, J. (2022). Severity of Anemia During Pregnancy and Adverse Maternal and Fetal Outcomes. *JAMA network open*, *5*(2), e2147046-e2147046.
58. Sobowale, O. I., Khan, M. R., Roy, A. K., Raqib, R., & Ahmed, F. (2022). Prevalence and Risk Factors of Vitamin B12 Deficiency among Pregnant Women in Rural Bangladesh. *Nutrients*, *14*(10), 1993.
59. Stangret, A., Skoda, M., Wnuk, A., Pyzlak, M., & Szukiewicz, D. (2017). Mild anemia during pregnancy upregulates placental vascularity development. *Medical Hypotheses*, *102*, 37-40.
60. Stangret, A.; Skoda, M.; Wnuk, A.; Pyzlak, M.; Szukiewicz, D. Mild anemia during pregnancy upregulates placental vascularity development. Med. Hypotheses 2017, 102, 37–40.
61. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
62. Tamura, T., & Picciano, M. F. (2006). Folate and human reproduction. *The American journal of clinical nutrition*, *83*(5), 993-1016.
63. Van Sande, H., Jacquemyn, Y., Karepouan, N., & Ajaji, M. (2013). Vitamin B12 in pregnancy: maternal and fetal/neonatal effectsA review. *Open journal of obstetrics and gynecology.-Irvine, CA, 2011, currens*, *3*(7), 599-602.
64. van Santen, S., Kroot, J. J., Zijderveld, G., Wiegerinck, E. T., Spaanderman, M. E., & Swinkels, D. W. (2013). The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. *Clinical chemistry and laboratory medicine*, *51*(7), 1395-1401.
65. Viteri, F. E. (1998). Prevention of iron deficiency. *Prevention of micronutrients deficiencies. Tools for policymakers and public health workers. Washington: National Academy Press*, 45-102.
66. Wahed, F., Latif, S. A., Uddin, M. M., Mahamud, M. M., Sarker, D., & Hossain, M. Z. (2007). Persistence of low serum iron and high total iron binding capacity in pregnant women. *Mymensingh Medical Journal: MMJ*, *16*(2), 132-136.
67. Walker, M. C., Smith, G. N., Perkins, S. L., Keely, E. J., & Garner, P. R. (1999). Changes in homocysteine levels during normal pregnancy. *American journal of obstetrics and gynecology*, *180*(3), 660-664.
68. Wojciechowska, M., Wisniewski, O. W., Kolodziejski, P., & Krauss, H. (2021). Role of hepcidin in physiology and pathophysiology. Emerging experimental and clinical evidence. *Journal of Physiology and Pharmacology: an Official Journal of the Polish Physiological Society*, *72*(1).
69. YILMAZ, Z., ÇALIŞKAN, C. S., GÜNGÖR, N. D., YILMAZ, M., ÇELİK, S., & ÇELİK, S. (2022). The effects of vitamin deficiencies in the first trimester on pregnancy outcomes. *Journal of Experimental and Clinical Medicine*, *39*(1), 121-124.
70. Yip, R. (2000). Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *The American journal of clinical nutrition*, *72*(1), 272S-279S.

71. Yu, C. K. H., Sykes, L., Sethi, M., Teoh, T. G., & Robinson, S. (2009). Vitamin D deficiency and supplementation during pregnancy. *Clinical endocrinology*, *70*(5), 685-690.
72. Zaman, B., Rasool, S., Jasim, S., & Abdulah, D. (2021). Hepcidin as a diagnostic biomarker of iron deficiency anemia during pregnancy. *The Journal of Maternal-Fetal & Neonatal Medicine*, *34*(8), 1288-1296.