Original Article

Evaluation of Hepcidin and Iron-Biochemical Parameters in Pregnant Women in Samarra City

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Abstract

Background: Some studies have shown that the hepcidin hormone is inhibited during pregnancy, as the results indicated that hepcidin is at lower levels during pregnancy than in a nonpregnant state, and it is assumed that this happens to ensure greater bioavailability of iron for the mother and fetus. **Objectives:** This study aimed to estimate hepcidin concentration and iron homeostasis with some biochemical variables for pregnant women. **Materials and Methods:** A total of 90 blood samples were collected. Of 90 blood samples, 69 from pregnant women, which were divided into three groups according to the three pregnancy periods (23 samples for each period), and 21 blood samples from nonpregnant women (control group), levels of hepcidin, ferritin, iron, hemoglobin, total binding capacity of iron, unsaturated capacity of iron, transferrin and percentage of transferrin saturation were estimated in the pregnant women and nonpregnant women groups. **Results:** The results indicated a decrease in the concentration of hepcidin, iron, ferritin, hemoglobin, and the percentage of transferrin saturation at a probability $P < 0.05$, while a rise was observed in the level of total iron-binding capacity, unsaturated binding capacity of iron, and transferrin concentration at a probability $P < 0.05$ in blood serums of pregnant women compared with nonpregnant women. The results showed significant correlations in the serums of the groups under study. **Conclusion:** The results showed a significant decrease in the concentration of each of the hormone hepcidin, iron, hemoglobin, ferritin, and the percentage of transferrin saturation, with a significant increase in the level of total iron-binding capacity, unsaturated binding capacity of iron, and transferrin concentration.

Keywords: Hepcidin, iron, pregnant women

Introduction

Pregnancy is a natural condition that the pregnant woman adapts to during an extended period from egg fertilization to childbirth. The pregnancy lasts about 9 months. During this period, the pregnant woman undergoes many physiological changes to keep pace with the process of growth and development in the fetus.[1] The growth of one or more fetuses occurs inside The mother's womb, which is known as the embryo during the first eight weeks of pregnancy, after which it is called the term fetus until birth.[2] The pregnancy period lasts approximately 40 weeks, and the pregnancy period can be divided into three periods of time, the first period starts from the beginning of pregnancy until 13 weeks this period is characterized by the fact that it represents the highest risk of miscarriage. The second period of pregnancy starts from 13 to 26 weeks, during which the fetus's growth and development occur. The third period starts from 26 to 40 weeks, and this period represents the beginning of the life of the organism.[3] Many hematological changes and biochemical parameters occur for women during pregnancy, and the changes begin around the fourth week of pregnancy to reach the highest level in weeks 28–32, and the value of these changes is higher than their value in nonpregnant women, and the value of blood plasma increases to reach about 40%–45%, and the mass of corpuscles increases. Red blood at a rate of 30%–35% due to the increase in the hormone erythropoietin,[4] and the

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volume of plasma increases gradually during pregnancy, and the increase is 50% in the 34th week of pregnancy. The increase is proportional to the weight of the child at birth,[5] the increase in the volume of blood plasma is significant in maintaining blood dynamics and helping in the distribution and delivery of nutrients to the tissues of the mother and fetus,[6] During pregnancy, a well-balanced diet rich in carbohydrates, vitamins, proteins, and minerals is essential.[7]

Hepcidin is a peptide hormone made in the liver and was discovered in 2000. This hormone was discovered as a mediator in natural immunity, as its level increases with inflammation and infection,[8] and it was detected for the first time in human urine and blood,[9] hepcidin regulates the body's iron negatively by binding to the cellular iron exporter ferroportin in target cells, like macrophages, enterocytes and making its internalization and deprivation, thus reducing iron efflux into plasma causing decrease serum iron level and an elevated in intracellular iron,[10] A recent study indicated that there is a similarity between the cytoplasmic tail, which is rich in the amino acid cysteine, of the COVID-19 virus protein, with the hepcidin protein present in humans and vertebrate animals.[11] Stimulating iron in the plasma and increasing its storage is associated with the production of the hormone hepcidin and thus hinders the absorption of iron from the diet. During infection, hepcidin plays a defensive role against various infections by depleting extracellular iron from the body.[12] Also, hepcidin is a hepatic bactericidal protein rich in cysteine,[13] Some studies showed that the hormone hepcidin is inhibited during pregnancy, as the results indicated that the hepcidin levels are lower during pregnancy than in nonpregnant states, presumably to ensure greater bioavailability of Iron to the mother and fetus.[14,15]

Materials and Methods

Study samples

A total of 90 blood samples were collected from pregnant and nonpregnant women, aged between 18 and 40 years, from the laboratories of Samarra General Hospital and one of the specialized clinics in Samarra City from November 25, 2022, to December 30, 2022. Sixty-nine blood samples were taken from pregnant women divided into three pregnancies (23 samples for each period) and 21 samples of nonpregnant women, representing a control group.

The study included the estimation of the level of hepcidin hormone using an assay kit prepared by the Chinese company SUNLONG by the enzyme-linked immunosorbent assay (ELISA) technique, Sandawich type,[16] and the estimation of the level of ferritin by the enzyme-linked immunosorbent adsorption (ELISA) test technique,[17] in addition to measuring the level of hemoglobin using the German Sysmex XP-300 device, which gives a complete picture of the blood,[18] as well as measuring the concentration of each of the iron supplied by the French company Biolabo,[19] and measuring the concentration of the total iron-related capacity (TIBC) and non-iron-binding capacity (UIBC) saturation,[19] transferrin concentration calculation[20] and transferrin saturation percentage.[19]

Statistical analysis

The statistical program (SPSS V. 25) was used, and the mean and standard deviation (SD) were determined, as well as the means for the group of pregnant women compared with nonpregnant women using the independent *t* test at a probability level of $P < 0.05$.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and consent form were reviewed and approved by a local ethics committee according to the document number 66 (including the number and the date on November 22, 2022) to get this approval.

Results

The levels of hepcidin, iron, hemoglobin, and ferritin were estimated in the blood serums of pregnant and nonpregnant women, and the results were as shown in Table 1.

Results in Table 1 show that the mean ± SD of the hepcidin concentration in pregnant women was (101.04 ± 13.23) ng/mL, while in nonpregnant women, it was (122.15 ± 13.63) ng/mL, evident from the results above, the level of hepcidin was significantly decreased ($P < 0.05$) in the pregnant women group compared with nonpregnant women.

Table 1: Mean ± SD of biochemical parameters in the pregnant and nonpregnant women

Parameters	Control, mean ± SD	Patients, mean ± SD	$P \leq 0.05$
Hepcidin (ng/mL)	122.15 ± 13.63	101.04 ± 13.23	$P < 0.0001$
Iron (µmol/L)	28.66 ± 3.48	20.39 ± 3.77	$P < 0.0001$
Hemoglobin	13.01 ± 0.58	11.96 ± 0.70	$P < 0.0001$
Ferritin (ng/mL)	17.97 ± 2.27	15.05 ± 2.78	$P = 0.015$

Table 2: Mean ± standard deviation of hepcidin, iron, hemoglobin and ferritin levels in blood serum of pregnant women divided over the three pregnancies, compared with nonpregnant women

Parameters	Control, mean ± SD	Patients, mean ± SD			$P \leq 0.05$
		First trimester	Second trimester	Third trimester	
Hepcidin (ng/mL)	122.15 ± 13.63	107.00 ± 10.78	104.96 ± 12.35	91.17 ± 10.83	$P < 0.001$
Iron (µmol/L)	28.66 ± 3.48	24.60 ± 1.58	19.60 ± 2.60	16.95 ± 1.79	$P < 0.001$
Hemoglobin	13.01 ± 0.58	12.24 ± 0.79	11.90 ± 0.55	11.73 ± 0.67	$P < 0.001$
Ferritin (ng/mL)	17.97 ± 2.27	16.96 ± 2.65	14.87 ± 2.67	13.45 ± 2.35	$P < 0.001$

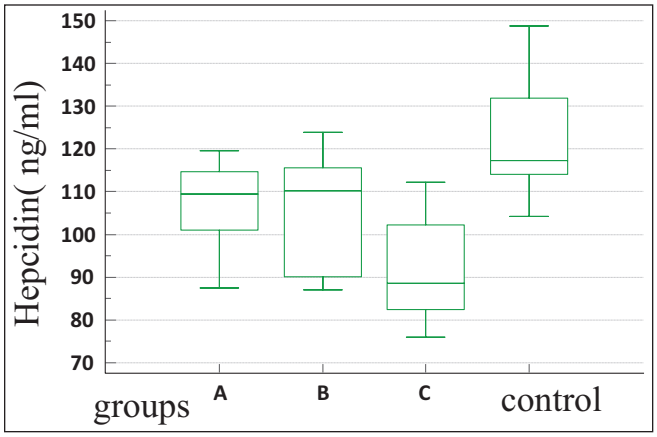

Figure 1: Hepcidin concentration in serum of two groups of pregnant and nonpregnant women, divided over the three pregnancies

Table 2 shows a significant decrease in concentration of hepcidin hormone during the three pregnancies compared to nonpregnant women. As shown in Figure 1, which shows hepcidin concentrations divided by the three pregnancies and nonpregnant women:

Results in Table 1 show that the mean ± SD of the iron concentration in pregnant women was (20.39 ± 3.77) µmol/L, while in nonpregnant women, it was (28.66 ± 3.48) µmol/L, evident from the results above, the iron concentration was significantly decreased ($P < 0.05$) in the pregnant women group compared to nonpregnant women.

Table 2 shows a significant decrease in concentration of iron during the three pregnancies compared to nonpregnant women. The results indicate that there is a significant difference in the concentration of iron between the three pregnancies, as shown in Figure 2, which shows iron concentrations divided over the three pregnancies and nonpregnant women:

Table 1 shows that the mean ±SD of the hemoglobin concentration in pregnant women was (11.96 ± 0.70), while in nonpregnant women, it was (13.01 ± 0.58). It is clear from the above results that the hemoglobin concentration was significantly decreased ($P < 0.05$) in the pregnant women group compared to nonpregnant women.

Table 2 shows a significant decrease in hemoglobin concentration during the three pregnancies compared to nonpregnant women, and the results indicate that there

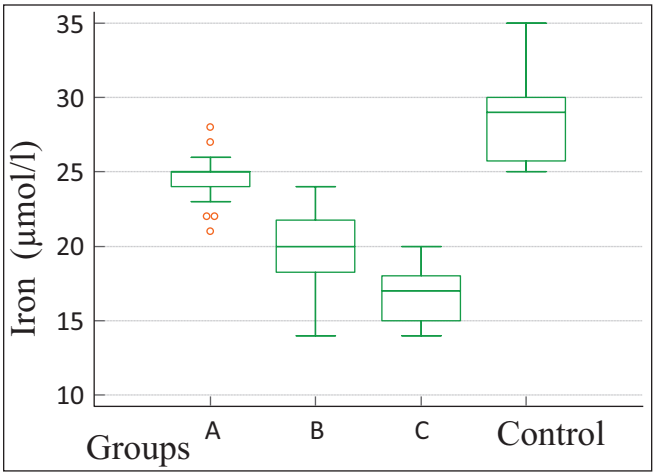

Figure 2: Iron concentration in serum of two groups of pregnant and nonpregnant women divided over the three pregnancies

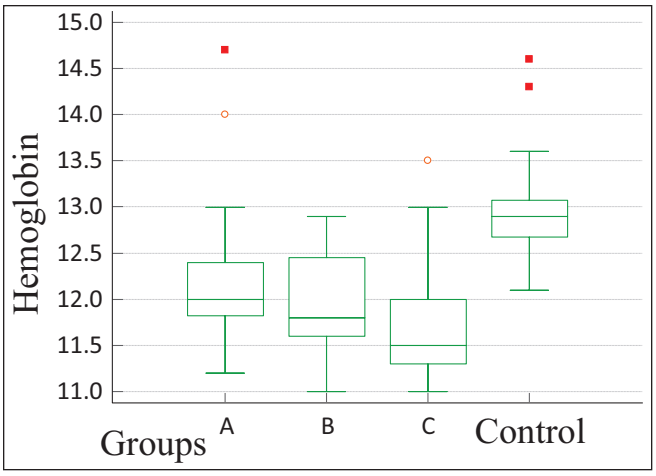

Figure 3: Hemoglobin concentration in the serum of two groups of pregnant and nonpregnant women divided over the three pregnancies

is no significant difference in hemoglobin concentration between the three pregnancies, as shown in Figure 3, which shows hemoglobin concentrations divided among the three pregnancies and nonpregnant women.

Table 1 shows that the mean ± SD of the ferritin level in pregnant women was (15.05 ± 2.78) ng/mL, while in nonpregnant women, it was (17.97 ± 2.27) ng/mL. It is clear from the above results that the ferritin concentration

was significantly decreased ($P < 0.05$) in the pregnant women group compared to nonpregnant women.

Table 2 shows a significant decrease in ferritin concentration during the second and third trimesters of pregnancy compared to nonpregnant women, with no significant decrease in ferritin concentration during the first trimester of pregnancy compared to nonpregnant women, as shown in Figure 4, which shows ferritin concentrations divided by the three pregnancies and nonpregnant women.

The concentration of total iron-binding capacity, unsaturated binding capacity, transferrin, and the percentage of transferrin saturation were measured in the serum of pregnant women compared to nonpregnant women, as shown in Table 3.

Table 3 shows that the mean ± SD of the TIBC level in pregnant women was (51.00 ± 5.19) µmol/L, while in nonpregnant women, it was (42.61 ± 3.30) µmol/L.

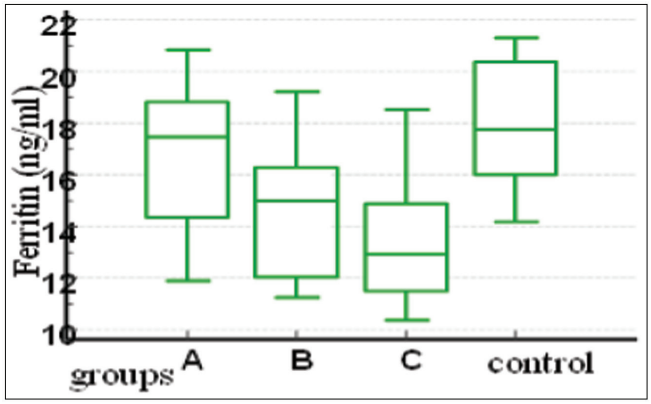

Figure 4: Ferritin concentration in serum of pregnant and nonpregnant women, divided over the three pregnancies

The mean ± SD of UIBC in pregnant women was (36.47 ± 4.46) mean ± SD, and in nonpregnant women, it was (30.38 ± 4.10) mean ± SD. It is clear from the above results that the level of TIBC and UIBC was significantly increased ($P < 0.05$) in the group of pregnant women compared with nonpregnant women.

Table 4 shows that there was an increment in TIBC and UIBC with progressing in pregnancy but statistically nonsignificant, whereas the results showed a significant increase in concentration of both TIBC and UIBC in serum of pregnant women compared to nonpregnant women as shown in Table 3.

Table 3 shows that the mean ± SD of transferrin concentration in pregnant women was (38.03 ± 4.79) µmol/L, while in nonpregnant women, it was (30.90 ± 3.22) µmol/L, as evident from the above results. The transferrin concentration was significantly increased ($P < 0.05$) in the group of pregnant women compared to nonpregnant women.

Table 4 shows that there was an increment in transferrin with progressing in pregnancy but statistically nonsignificant, but in general the results show a significant increase in concentration of transferrin in serum of groups of pregnant women compared to nonpregnant women as shown in Table 3.

Table 3 shows that the mean ± SD of the transferrin saturation percentage in pregnant women was (34.89 ± 8.74)%, while in nonpregnant women, it was (59.57 ± 4.77)%. It is clear from the above results that the percentage level of transferrin saturation was significantly decreased ($P < 0.05$) in the group of pregnant women compared to nonpregnant women.

Table 3: Mean ± SD of biochemical parameters in the pregnant and nonpregnant women

Parameters	Control mean ± SD	Patients mean ± SD	$P \leq 0.05$
TIBC (µmol/L)	42.61 ± 3.30	51.00 ± 5.19	$P < 0.0001$
UIBC (µmol/L)	30.38 ± 4.10	36.47 ± 4.46	$P < 0.0001$
Transferrin (µmol/L)	30.90 ± 3.22	38.03 ± 4.79	$P < 0.0001$
TSAT %	59.57 ± 4.77	34.89 ± 8.74	$P < 0.0001$

TIBC: total iron-binding capacity, UIBC: unsaturated iron-binding capacity, TSAT %: transferrin saturation percentage

Table 4: Mean ± standard deviation of total iron-binding capacity, unsaturated iron bending capacity, transferrin saturation, and percentage of transferrin saturation in the serum of pregnant women divided over the three pregnancies compared with nonpregnant women

Parameters	Control mean ± SD	Patients mean ± SD			$P \leq 0.05$
		First trimester	Second trimester	Third trimester	
TIBC (µmol/L)	42.61 ± 3.30	49.47 ± 5.74	50.21 ± 5.00	53.30 ± 4.10	$P < 0.001$
UIBC (µmol/L)	30.38 ± 4.10	35.43 ± 4.23	36.04 ± 4.85	37.95 ± 4.05	$P < 0.001$
Transferrin (µmol/L)	30.90 ± 3.22	36.58 ± 4.87	37.22 ± 4.15	40.29 ± 4.68	$P < 0.001$
TSAT %	59.57 ± 4.77	41.91 ± 3.91	35.86 ± 9.53	26.91 ± 3.32	$P < 0.001$

TIBC, total iron-binding capacity, UIBC: Unsaturated iron-binding capacity, TSAT %: transferrin saturation percentage

Table 5: The correlations between iron and other parameters in control and patients

Parameters	Iron	
	Control	Patients
TIBC	0.752	0.179
UIBC	0.742	0.176
Transferrin	0.642	0.169
TSAT %	0.646	0.660

Table 6: The correlations between transferrin and other parameters in control and patients

Parameters	Transferrin	
	Control	Patients
TIBC	0.938	0.997
UIBC	0.935	0.994
TSAT %	-0.098	-0.525

Table 4 shows that there is a significant difference ($P < 0.001$) in transferrin saturation percentage between the three pregnancies, and the results also show a significant decrease in the percentage of transferrin saturation in serum of groups of pregnant women compared to nonpregnant women as shown in Table 3.

The correlations were also studied between iron and other parameters in the groups under study, as shown in Table 5.

The results in Table 5 shows that the correlation of Iron with TIBC, UIBC, transferrin, and TSAT % in the control group was a significant positive correlation, as the value of the correlation coefficient were ($r = 0.752$), ($r = 0.742$), ($r = 0.642$), and ($r = 0.646$), respectively, whereas there was no correlation between iron and TIBC, UIBC, and transferrin in the patients group, but results showed positive correlation between iron and TSAT % as the value of the correlation coefficient was ($r = 0.660$).

The correlations between transferrin and other parameters in the groups under study were studied as shown in Table 6.

The results in Table 4 shows a significant positive correlation of transferrin with TIBC and UIBC in control and patients, the value of the correlation coefficient of TIBC and UIBC in control and patients, were ($r = 0.938$), ($r = 0.935$), ($r = 0.997$), and ($r = 0.994$), respectively, whereas there was no correlation between transferrin and TSAT % in control group, while was negative correlation between transferrin TSAT % in patients group, as $r = -0.525$.

Discussion

The hepcidin hormone is a central regulator of iron balance in the body.[21] The availability of iron is critical during pregnancy for both the mother and the fetus,[22] and during pregnancy, hepcidin is associated with iron, but has nothing to do with inflammation during pregnancy.[23] The results indicated in this study revealed a gradual decrease in the concentration of the hepcidin in the blood serum of pregnant women from the first trimester to the third and last trimester of pregnancy, compared with nonpregnant women, and this was in agreement with a study conducted by Van Santen *et al.*,[24] and Hassan.[25] Hepcidin concentrations in pregnant women are lower than in healthy, nonpregnant women, and hepcidin levels decrease as pregnancy progresses, with the lowest hepcidin levels observed in the third trimester.[24,26]

Iron is essential for the function of all cells through its roles in oxygen delivery, electron transport, and enzymatic activity. Iron requirements during pregnancy increase dramatically as the mother's blood volume expands and the fetus grows and develops. Thus, pregnancy is a state of impending or existing iron deficiency, which may be challenging to diagnose due to limitations of commonly used biomarkers such as hemoglobin and ferritin concentrations.[27] The results of this study agreed with a study conducted by Raza *et al.*,[28] which indicated a gradual decrease in the concentration of iron in the serum of pregnant women from the first trimester to the second trimester and then to the third trimester compared to nonpregnant women.

The primary function of hemoglobin in mammals is to transport oxygen from the lungs to the tissues.[29] Low hemoglobin concentrations during pregnancy can be associated with an increased maternal and perinatal mortality risk and low birth weight or size.[30] The results of this study agreed with a study conducted by Akinbami *et al.*,[31] that indicated a decrease in hemoglobin concentration in the serum of pregnant women compared to nonpregnant women.

Serum ferritin levels reflect the amount of iron stored.[32] The results of this study agreed with the study conducted by Perlas *et al.*,[33] as they showed that ferritin concentration decreases in the blood serums of pregnant women compared to nonpregnant women, where a slight decrease occurs in the first trimester of pregnancy compared to healthy subjects in when a significant decrease in ferritin concentration occurs in the second and third trimesters of pregnancy. Whereas Kaneshige[34] indicated in his study that blood ferritin levels increased significantly in the first trimester of pregnancy compared to nonpregnant women. In contrast, blood ferritin levels decreased significantly during the second and third trimesters of pregnancy. So, the same results that ferritin in the second and third trimester is lower than first trimester.

TIBC total iron-binding capacity indicates the maximum amount of iron needed to saturate plasma or serum transferrin TRF, an essential iron transport protein,[35] UIBC increases when iron stores are depleted, UIBC is

more accurate than total iron-binding capacity TIBC and transferrin saturation in the diagnosis of empty iron stores.[36] The results of this study agreed with a study conducted by AL-Hamdani,[37] which indicated an increase in the levels of both TIBC and UIBC in the pregnant women compared to with nonpregnant women.

Serum transferrin is a sensitive indicator of iron deficiency in inflammatory conditions and in anemia of chronic disease because its concentration is not affected by the acute-phase response.[38] The results of this study agreed with a study conducted by Talabani,[39] which indicated that transferrin concentrations increase to a maximum extent during the third trimester of pregnancy compared to healthy subjects.

As for the percentage of transferrin saturation, the results indicated a decrease in the blood serum of pregnant women gradually from the first trimester to the third trimester, and these results agreed with the results of the study conducted by Noshiro *et al*.[40]

The results of the study showed that there is a positive correlation of Iron with TIBC, UIBC, and transferrin in groups of nonpregnant women, with no correlation in pregnant groups, whereas the results showed a positive correlation between iron and TSAT % in pregnant and nonpregnant groups.

The results of the study showed that there is a positive correlation of transferrin with TIBC and UIBC in pregnant and nonpregnant women, with a negative correlation between transferrin and TSAT % in pregnant group.

Conclusion

The results showed a significant decrease in the level of the hormone hepcidin, iron concentration, hemoglobin, ferritin, and the percentage of transferrin saturation, accompanied by a rise in the level of total iron-binding capacity, the unsaturated capacity of iron, and the level of transferrin in the pregnant women compared with nonpregnant women. Hepcidin evaluation is recommended for pregnant women with Gestational diabetes and pregnant women with Preeclampsia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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