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Original article

Study of the hepcidin level in pregnant women with and without anemia

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Abstract. *Background and aim:* Currently, a wide range of laboratory markers characterizing iron metabolism is available to clinicians. However, they are mainly limited by determining the hemoglobin content, iron concentration and erythrocyte morphology, which makes it impossible to differentiate iron deficiency anemia from other hypochromic anemia forms. The study aimed to evaluate the diagnostic value of hepcidin as a ferrokinetics marker under the development of anemia during pregnancy. *Methods:* The study included 60 pregnant women (average age 26.0±0.74 years). They were divided into two groups. The participants of the Main group had hypochromic microcytic anemia with the reference values of the red blood cells 4.15 million/μL and the hemoglobin level 105 g/L. The reference values of these marks in the Control group were 4.54 million/μL and 124.50 g/L respectively. *Results:* Low levels of Fe, ferritin, and hepcidin were detected in pregnant women with anemia. The serum hepcidin concentration of women with anemia was 0.55 ng/mL. The anemia-diagnosed group had a reduced ferritin level by 32% (4.5 ng/mL). This point for the Control group was 14.0 ng/mL. A positive moderate relationship was indicated for hepcidin and Fe (r=0.39; P=0.05). At the same time, a negative moderate relationship (r=-0.56; P=0.05) was found between hepcidin and ferritin. *Conclusions*: Data from pregnant women with anemia showed hypochromic microcytic anemia with a serum ferritin level of 4.5 ng/mL and hepcidin level of 0.55ng/mL. The present research furtherly supports the valid role of hepcidin in the diagnosis of iron deficiency anemia in pregnant women. (www.actabiomedica.it)

Key words: Anemia, pregnancy, hepcidin

Introduction

Anemia, a decrease in the number of red blood cells (RBC) or hemoglobin concentration (Hb) in the blood, is a serious pathological condition. It is characterized by such symptoms as fatigue, weakness, dizziness, drowsiness, and dyspnea. Children and pregnant women are at risk group for the disease. The World Health Organization (WHO) document showed that about 38% (32 million) of pregnant women are anemic in the word, 75% of all anemia diagnosed were due to iron deficiency (1, 2). Women in third trimester pregnancy are more likely risky to develop anemia as compared to first and second trimester (2, 3). In pregnant women, iron deficiency anemia is associated with adverse reproductive outcomes such as preterm delivery, low-birth-weight infants, and decreased iron stores for the baby, which may lead to impaired development (1, 4).

Various studies had examined multiple aspects upsetting anemia in pregnancy. It has been found that hemoglobin values <110 g/L in the first and third trimesters, and <105 g/L in the second trimester, may point to an anemic condition that should be further clarified. Among the most important differential diagnoses for anemia during pregnancy are: (a) iron-deficiency anaemia; (b) hemoglobinopathies (thalassaemia, sickle cell anaemia); (c) infections, e.g malaria (d) parassitosis; (e) malnutrition and chronic diseases (5).

The human body contains 5-6 g of iron, with the main amount in hemo- and myoglobin. The binding of O_2 in the erythrocyte is not random: the attachment of each oxygen molecule to one of the sites facilitates this process for the remaining active sites within the cell. The binding of two O_2 molecules facilitates the binding of Fe^{2+} with the third oxygen molecule. The iron molecule is the key one in providing the body cellwith oxygen. The study of its metabolism is important for the prevention and treatment of iron deficiency anemia (6-8).

Ferritin, a divalent metal transporter (duodenal metal transporter), ferroportin, a duodenal cytochrome B, hephaestin, an iron-sensitive elements and iron regulatory protein, and hepcidin, a key iron regulating hormone, are involved in the process of metabolism (absorption and regulation of Fe^{2+} homeostasis in blood cells) (8).

Ferritin, as a depot of iron ions, in a molecule can contain up to 4000 iron atoms. Ferritin level decreasing can be considered a symptom of iron deficiency anemia (9-10). According to the WHO, the ferritin concentration fairly accurately reflects the iron stores in the body, but only in the absence of inflammation (i.e., in apparently healthy individuals). So, the determination of the serum ferritin concentration in apparently healthy patients is an important marker of ferrokinetics (11). However, there are no clear norms and mechanisms for ferritin correlation in pregnancy. There is evidence of changes in its concentration in the blood serum during this period. Ferritin concentration steadily decreases in the second trimester and continues to decrease in the third trimester. Bobrov et al. indicate an 80% depletion of the iron depot compared with the first trimester: serum ferritin concentration in the third trimester is 20% compared to the first trimester of pregnancy (12).

Another important regulator of Fe^{2+} metabolism is the iron-regulating hormone hepcidin. This protein inhibits ferroportin by limiting the transport of iron into the cell by the villi of the small intestine and blocking the release of iron from macrophages. This leads to a violation of iron homeostasis, iron deficiency, and anemia (13).

Currently, active work is being carried out to improve the diagnosis and differential diagnosis of the anemic syndrome, as well as to search for new ferrokinetics markers and optimize the existing laboratory diagnostic algorithm.

The study aimed to evaluate the diagnostic value of hepcidin as a ferrokinetics marker under the development of anemia during pregnancy.

Materials and methods

Research design

A clinical prospective controlled study has been conducted. Within the study, two observation groups were randomly formed. The Main group (n = 30) included pregnant women whose Hb level and erythrocyte count were within the normal ranges. Control group (n = 30) included pregnant women whose Hb level and erythrocyte count were within the normal ranges. The groups were comparable in age. The average patients' age was 26.0 ± 0.74 years. All of the pregnant were enrolled and controlled in the Family Planning Center and the Obstetrics and Gynecology Department of the Aktobe Medical Center, Aktobe, Kazakhstan.

The women in the second pregnancy trimester were included in the study. A serum ferritin concentration <30 ng/mL together with an Hb concentration <11 g/dL during the 1st trimester, <10.5 g/dL during the 2nd trimester, and <11 g/dL during the 3rd trimester are diagnostic for anemia during pregnancy. Iron deficiency anemia during pregnancy poses a number of maternal and fetal problems, including premature birth, intrauterine developmental retardation, placental problems, and a decrease in newborn iron storage.

In the study, we included only women in the second pregnancy trimester without secondary causes of anemia.

The research has been conducted according to the ethical principles of the Helsinki Declaration and the local Bioethics Committee of West Kazakhstan Marat Ospanov Medical University was requested (protocol no. 5, March 13, 2020).

Studied parameters

Hematology markers were analyzed by us to characterize the condition of women in the studied groups (Table 1).

According to WHO, the criterion for anemia in the second trimester was a decrease in hemoglobin level (HB) < 110 g/L, Red Blood Cells (RBC) < 3.8 million/μL, and hematocrit (HT) < 33% (14).

Statistical analysis

The results were analyzed by methods of descriptive, correlation, nonparametric statistics using the Mann-Whitney test to determine differences in values for small groups. The difference was considered statistically significant at P: >0.05. The Statistics 10 software package was used to process the study results.

Results

Similar studies of hematological parameters were conducted for all observed patients to determine the particularities of iron metabolism. The following iron metabolism markers were studied for all subjects: serum iron (Fe), total serum iron-binding capacity (TIBC), latent serum iron-binding capacity (LIBC), and serum ferritin (SF) were studied; as well as the level of hepcidin was determined by direct enzyme-linked immunosorbent assay (ELISA). The obtained indicators are presented in Table 2.

Analysis of the obtained laboratory indicators showed a decrease in MCH (25 pg) and MCV (82 fl) and an increase in RDW (14.6%) compared to the control group (28.7 pg; 88.3 fl and 13.3%, respectively).

Table 1. The markers examined during the study – reference values and indicators of anemia.

No	Tested markers	Reference values	Anemia marker
Morphological Parameters*			
1	Hematocrit, %	33-45.0	<33
2	Hemoglobin, g/L	110-155	<110
3	RBC number, million/μL	3.8-5.10	<3.8
4	Mean corpuscular volume, fL	80.0-100	<80
5	Red cell distribution, %	11.6-14.8	>14.8
6	Mean corpuscular volume, pg	27.0 -34.0	<27
7	Mean Corpuscular Hemoglobin Concentration, g/dL	34.0-36.0	<34
Biochemical Parameters*			
1	Fe concentration, μmoll/L	12.5-32	<12.5
2	Latent iron binding capacity, μmoll/L	24.2-70.1	>70
3	Total iron binding capacity, μmoll/L	41-77	>77
4	Ferritin, ng/mL	10-120	<10 iron deficiency anemia, >120 anemia of the chronic inflammation
5	Hepcidin**, ng/mL	1.49-41.46	<1.49

*The study was carried out in INVITRO laboratory (Aktobe); **Determination of hepcidin was carried out in the laboratory of Moscow.

Table 2. Laboratory diagnostic indicators.

Tested parameter	Main group			Control group			Ref. value	*P*
	Me	Q1	Q3	Me	Q1	Q3		
The age, years	26.00	25.00	28.50	26.00	25.00	31.00		0.75
HT, %	34.35	31.60	35.45	39.10	38.00	40.30	40	<0.001
Hb, g/L	105.00	95.00	113.00	124.50	120.00	131.00	105	<0.001
RBC, million/μL	4.15	3.80	4.40	4.54	4.21	4.65	4.45	0.003
MCV, fl	82.50	74.75	87.45	88.30	85.20	90.80	90.5	0.001
RDW, % (anisocytosis)	14.60	13.25	16.50	13.30	12.70	14.00	13.2	0.01
MCV, pg	25.15	22.10	27.95	28.70	26.90	29.90	30.5	0.003
MCHC, g/dL	30.65	29.00	31.90	31.90	31.30	32.80	34	0.002
Fe, μmol/L	6.30	4.25	9.55	12.30	8.79	17.58	21.7	0.00008
Ferritin, ng/mL	4.50	3.00	7.50	14.00	7.00	31.00	65	0.00005
LIBC, μmol/L	62.00	52.00	70.00	47.00	40.00	57.00	34	0.003
TIBC, μmol/L	68.20	62.35	77.00	61.30	54.00	68.00	60.55	0.05
Hepcidin, ng/mL	0.56	0.37	0.89	2.36	0.81	3.41	21.47	0.0004

HT – Hematocrit; Hb - Hemoglobin; LIBC – Latent iron binding capacity; MCV - Mean Corpuscular Volume; Me – median of the tested parameter; MCHC - Mean Corpuscular Hemoglobin Concentration; Q1 – the lowest level in the tested group; Q3 – the highest level of the parameter in the tested group; RBC – Red Blood Cells; RDW - Red Cell Distribution Width; TIBC - Total iron binding capacity

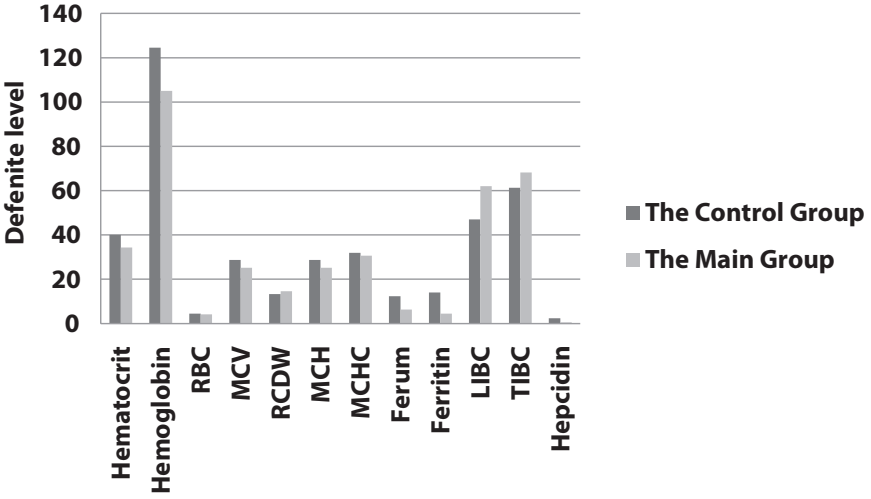

Figure 1. The hematological study results in the Main and Control groups.

We can note that in pregnant women with anemia were fixed a decrease in serum Fe, ferritin and hepcidin levels (6.2 μmol/L, 4.5 ng/mL and 0.55 ng/mL) compared to the Control group (12.2, 14.0 and 2.3, respectively).

Figure 1 allows us to visualize the difference between the obtained results in our study.

Figure 1 shows the difference in the hemogram indicators of the Main group: reduced hemoglobin levels (Hb), decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and increase in red blood cell distribution width (RDW). The decrease in serum Fe, SF, and hepcidin and the increase in LIBC and TIBC were detected.

Analysis of the concentrations of iron, ferritin, and hepcidin in the blood serum

Analysis of the results showed a decrease in serum Fe, ferritin, and hepcidin in pregnant women with anemia, which were 6.2 μmol/L, 4.5 ng/mL, and 0.55 ng/mL compared with the Control group with the indicators of 12.2, 14.0 and 2.3 ng/mL, respectively. Serum hepcidin concentrations are often undetectable or very low in iron deficiency. A hepcidin level of 2 ng/mL and/or a serum ferritin level ≤ 10 ng/mL can be considered an useful indicator of iron deficiency (15,16).

Additionally, a correlation analysis between the indicators of hepcidin and serum Fe was carried out. As a result, a positive moderate relationship was established (r=0.39; P=0.05) (Figure 2).

Further analysis showed a negative moderate relationship (r=-0.56, P=0.05) between hepcidin and ferritin (Figure 3).

The low levels of serum Fe, ferritin and hepcidin can be explained by a feedback mechanism. Decreased intestinal iron absorption with the gradual depletion of the iron transport pool causes inhibition of hepcidin synthesis in the liver. Iron absorption by enterocytes and its release from tissue macrophages is restored by the feedback principle (8, 13, 17).

Discussion

The result of the observational study showed a decrease (deficiency) in iron concentration of the

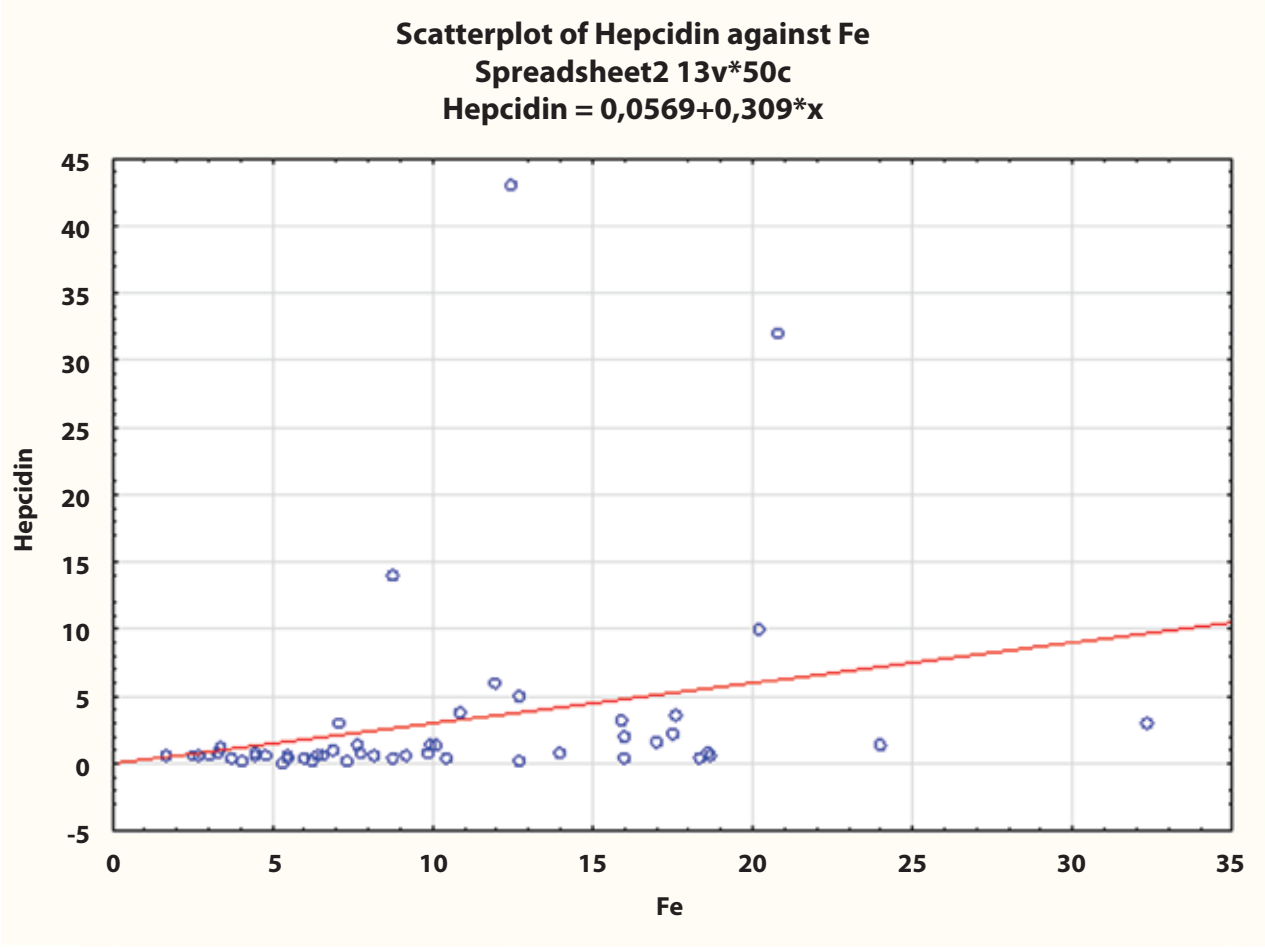

Figure 2. Correlation between hepcidin concentration and serum Fe^{2+} ions in pregnant women.

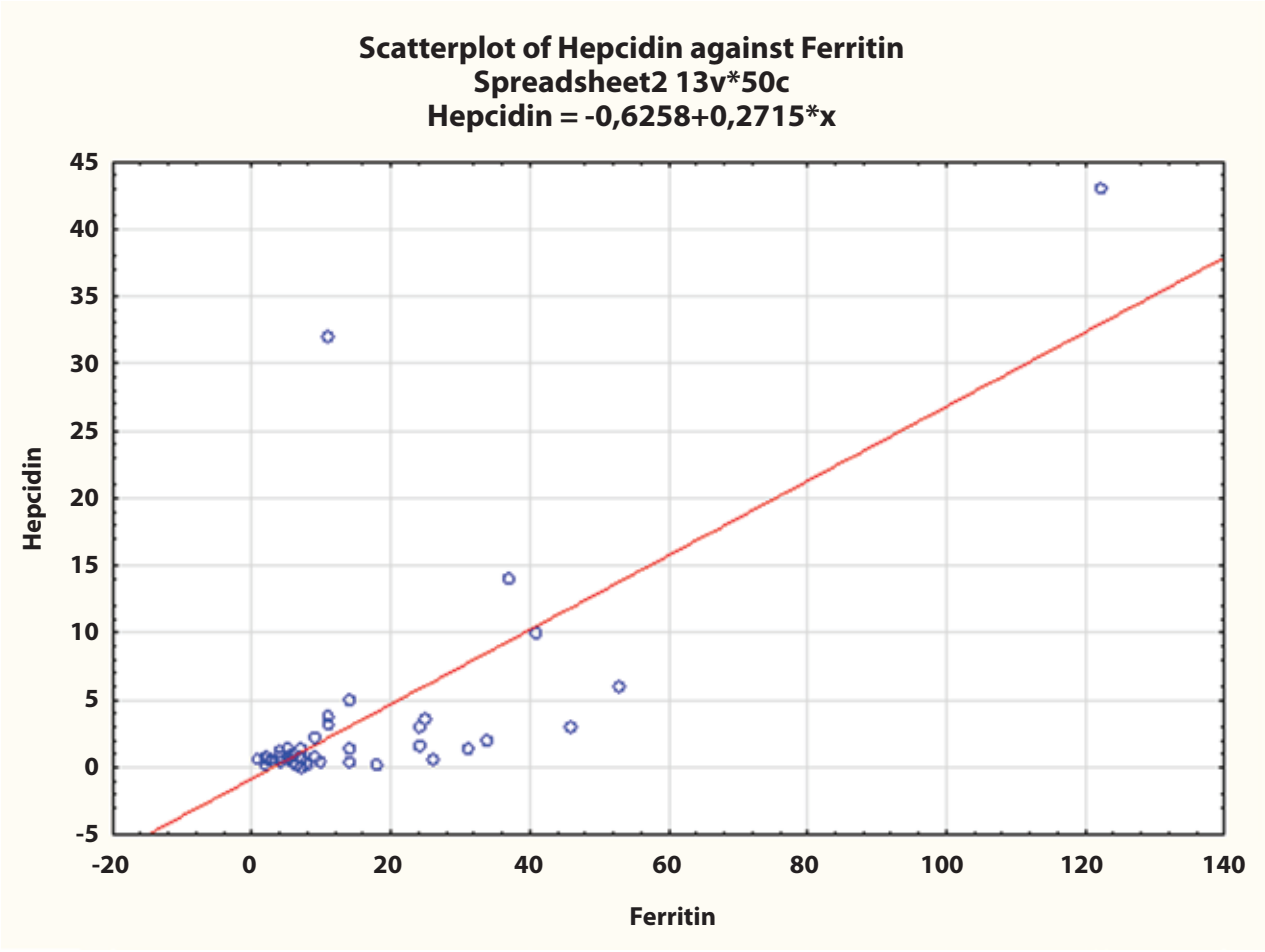

Figure 3. Visualization of correlation analysis of hepcidin and SF concentrations pregnant women.

examined women both in the Main and in the Control group. Analysis of specific biologically active components actively involved in iron metabolism in the body showed a decrease in SF and hepcidin concentrations. Hepcidin and SF levels in both groups were low: 3.2 and 14.3 ng/mL, respectively, compared with the average reference values of 21.4 and 65.0 ng/mL.

Ferritin is in almost all organs and body tissues. It is used like an iron donor by cells that need it. Ferritin decrease can be detected long before the onset of iron deficiency symptoms. So, the testing of the SF level can be used to diagnose iron deficiency anemia in time (10-12). Analysis of SF level in pregnant women showed its low indicator in patients with anemia (4.5 ng/mL at a reference value of 10-120 ng/mL). Comparison with the Control group showed a decrease to 32% (14.0 ng/mL).

The majority of authors consider the SF concentration in the range of 15-150 ng/mL to be the norm for women of reproductive age and pregnant women (18,19). However, we have no explicit norms for SF level for the pregnant women and persons with the mandatory determination of inflammatory markers. There are data about its concentration in a range of 15-50 ng/mL and reference values for pregnant women within 10-68 ng/mL. Its level of less than 12-15 ng/mL can be detected as a sharp deficiency of iron stores. But physicians should be aware that SF is also an acute phase reactant and may be in norm, even elevated, under inflammatory conditions despite the presence of

anemia, and in such cases, confirmation of the diagnosis may require additional tests (19-22).

The hepcidin concentration is a clear laboratory marker that allows us both to assess the anemia development and to predict the effectiveness of oral ferrotherapy. A hepcidin level of 2 ng/mL or less indicates an ineffective course of anemic condition correction in pregnant women (16).

Determination of serum hepcidin concentration in women during pregnancy showed hepcidin's low level in patients with anemia (0.56 ng/mL), while for the Control group this indicator was at the level of 2.36 ng/mL. We can see the differences in the levels of the tested groups but have to note the iron depletion in all tested pregnant women, as indicated by hepcidin levels that were going down in both groups. This can be explained by the physiological mechanisms of hepcidin synthesis by hepatocytes in response to elevated blood iron levels to prevent iron ion overload (8, 23). However, hepcidin not only blocks the process of iron absorption in the intestine but also slows down erythropoiesis. A violation of hepcidin synthesis can cause both anemias (at its high concentrations – with hepcidin-producing adenomas, anemia of chronic disease) and ferrotoxic conditions (in case of blocking its synthesis – with ineffective erythropoiesis) (24).

The correlation analysis between hepcidin and ferritin was carried out. Its results showed a negative moderate relationship between the studied parameters (r=-0.56; P=0.05) (Figure 3).

The data are comparable with the results of Peltec (10). He noted that almost all pregnant women (out of 134 tested) had depleted iron stores and developed iron deficiency before delivery. Ferritin control is one of the important markers of the pre-anemic condition since the development of Fe^{2+} deficiency will first of all be accompanied by a decrease in SF concentration, then serum iron, and only then by hemoglobin (9, 10).

Monitoring of ferritin and hepcidin levels as ferrokinetics markers connected with common parameters (RBC, hemoglobin, and serum iron levels) allows practitioners both to differentiate anemic conditions in pregnant women and may improve the targeting of iron supplementation programs in resource-limited countries, though the hepcidin's testing high costs may limit its use as well as its need for standardization.

Conclusions

Currently, iron deficiency anemia occupies a leading place in the structure of anemic conditions. Literature data indicate fundamental changes in iron metabolism understanding and a significant revision of the importance of iron-binding proteins in the general biological processes. Hepcidin is recognized by many researchers as a key iron regulatory hormone. Its level can indicate the anemia type. Hepcidin is decreased in iron deficiency anemia and increased in chronic inflammation anemia, which is associated with the inhibition of ferroportin by hepcidin protein.

Data from the study in pregnant women with anemia showed hypochromic microcytic anemia with a ferritin level of 4.5 ng/mL and hepcidin level of 0.55 ng/mL. Iron deficiency was detected in the examined women both in the Main and in the Control group.

Further studies to determine hepcidin levels in pregnant women with and without anemia may provide clinicians with new data to develop mechanisms of therapeutic action.

Practical applications

Determination of hepcidin in pregnant women with anemia can expand anemia pathogenesis understanding; provide additional opportunities for the diagnosis and differential diagnosis of anemia. The decrease in ferritin and hepcidin levels in the Control group may require further studies.

Limitations and strengths of the study

We studied the markers of blood ferrokinetics in pregnant women in the second trimester because of their representativeness. We have not taken into account the ecological (geochemical) parameters of the region. The present research furtherly supports the valid role of hepcidin for the diagnosis of iron deficiency anemia in pregnant women.

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Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Ethical Statement: Ethical Committee of the West Kazakhstan Marat Ospanov Medical University has considered and approved the presented research as conducted in Ethical principles (protocol no. 5 from 13.03.205).

Author Contributions Statement: Raisa Aringazina has been the main in the conception and research design creation. Ms. Aringazina has made the critical analysis and final reviewing of the manuscript. Aigul Mussina, Nurgul Zholdassova, Nazgul Seitmaganbetova and Gulnara Gubasheva have conducted the research; collect the research data, made it procession, and formed the manuscript. All of the authors have read and approved the final variant of the manuscript before its presentation in the Edition.

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