

# The Levels of Hepcidin and Erythropoietin in Pregnant Women with Anemia of Various Geneses

Dmitriy Vazenmiller\*, Olga Ponamaryova, Larisa Muravlyova, Vilen Molotov-Luchanskiy, Dmitriy Klyuyev, Riszhan Bakirova, Zhanna Amirbekova

*Karaganda State Medical University, Karaganda, Kazakhstan*

## Abstract

**Citation:** Vazenmiller D, Ponamaryova O, Muravlyova L, Molotov-Luchanskiy V, Klyuyev D, Bakirova R, Amirbekova Z. The Levels of Hepcidin and Erythropoietin in Pregnant Women with Anemia of Various Geneses. Open Access Maced J Med Sci. <https://doi.org/10.3889/oamjms.2018.471>

**Keywords:** Pregnant women; Anemia; Erythropoietin; Hepcidin

\***Correspondence:** Dmitriy Vazenmiller. Karaganda State Medical University, Karaganda, Kazakhstan. E-mail: stop\_@mail.ru

**Received:** 03-Oct-2018; **Revised:** 02-Nov-2018; **Accepted:** 03-Nov-2018; **Online first:** 23-Nov-2018

**Copyright:** © 2018 Dmitriy Vazenmiller, Olga Ponamaryova, Larisa Muravlyova, Vilen Molotov-Luchanskiy, Dmitriy Klyuyev, Riszhan Bakirova, Zhanna Amirbekova. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

**Funding:** This research did not receive any financial support.

**Competing Interests:** The authors have declared that no competing interests exist.

**AIM:** The purpose of the present research was to study the content of erythropoietin and hepcidin in serum in pregnant women with iron deficiency anaemia and anaemia of chronic inflammation.

**METHODS:** The authors examined 98 pregnant women who were observed in LLP (Regional obstetric-gynaecological centre) in Karaganda. The including criteria for pregnant women in the study was the informed consent of the woman to participate in the study. Exclusion criteria were oncological diseases, HIV-infection, tuberculosis, severe somatic pathology, mental illness, drug addiction. The design of the study was by the legislation of the Republic of Kazakhstan, international ethical norms and normative documents of research organisations, approved by the ethics committee of the Karaganda State Medical University.

**RESULTS:** As a result of the study, it was determined that the content of erythropoietin and hepcidin in pregnant women with anemias of different genesis varies ambiguously. In the main group of pregnant women with IDA, the erythropoietin content rises, and the hepcidin level decreases. In pregnant women with ACI, on the contrary, the level of hepcidin increases, and in one subgroup it is significant. However, in pregnant women with IDA and anaemia of chronic inflammation, there is a subgroup of women in whom erythropoietin is either comparable with hepcidin, or their changes are of opposite nature.

**CONCLUSION:** The authors concluded that the obtained data indicate ambiguous changes in the level of erythropoietin and hepcidin in pregnant women with anaemias of various origins. In all likelihood, there are still unaccounted factors affecting the content of these protein-regulators of iron metabolism, which require further definition and interpretation in anaemia of pregnant women.

## Introduction

Anaemia of pregnant women continues to be one of the main problems of obstetrics and gynaecology. The multifactorial nature of the development of anaemia in pregnant women (AP), the complexity of pathogenesis creates problems for determining the criteria for diagnosing the aetiology of anaemia and, subsequently, for adequate therapeutic correction. Traditional anaemia biomarkers are not always sufficiently informative to determine the aetiology of anaemia in pregnant women. This significantly complicates the implementation of preventive or therapeutic interventions to correct the violation of iron metabolism. A convincing opinion was expressed that in some cases, the appointment of iron preparations to pregnant women with anaemia of chronic inflammation can induce oxidative stress and

promote the growth of bacteria, thereby exacerbating the complications of pregnancy, i.e. anaemia of medium degree is a protective mechanism against bacterial and fungal pathogens [1] [2].

Currently, the main emphasis was made on the study of erythropoietin and hepcidin to deepen the understanding of the state of iron metabolism in particular and erythron in general in the body of pregnant women (including with AP).

Erythropoietin is a hormone, synthesised in the kidneys and perisinusoidal liver cells (mainly in the embryonic and perinatal periods). It is a glycoprotein with a molecular weight of 34 kDa. The secretion of erythropoietin in the blood is regulated by the body oxygen regime [3]. Synthesis of erythropoietin has no humoral or neural regulation. The production of erythropoietin depends only on the oxygen content and is controlled by the feedback principle.

The main function of erythropoietin is the regulation of erythropoiesis [4]. The intensity of the formation of new erythrocytes in the bone marrow depends on the level of endogenous erythropoietin in the plasma. Inadequate to hypoxia levels, the development of own erythropoietin can lead to the occurrence of anaemia. Under normal conditions, in response to a decrease in oxygenation of tissues and cells, there is an increase in the synthesis of erythropoietin by the kidneys. The isolated hormone interacts in the bone marrow with specific receptors on the surface of the progenitor cells of erythrocytes, which stimulates their proliferation and differentiation and, as a result, increases the concentration of haemoglobin.

Also, erythropoietin induces other effects, including increases systemic blood pressure, increases iron absorption by inhibiting hepcidin activity [5]. Erythropoietin also has a cytoprotective effect on the brain, kidney and heart cells by limiting apoptosis, oxidative stress, and has anti-inflammatory activity [6].

The erythropoietin concentration increases in 2-4 times at the developing physiological anaemia of pregnant women [7]. According to Erdem et al., [8], a low concentration of haemoglobin and ferritin with an increase in serum erythropoietin content was noted in pregnant women with anaemia. A similar view that anaemia during pregnancy induces the secretion of erythropoietin in response to a low content of haemoglobin and ferritin is expressed in a later study of Ervasti M. et al., [9]. It should also be noted that studies have appeared on the use of erythropoietin as a therapeutic agent in anaemia of pregnant women [10].

At the moment about 20 regulatory molecules are known to control this complex process. Over the past few years, the role of hepcidin as a key regulator of iron metabolism has been actively discussed.

Hepcidin, a peptide hormone consisting of 25 amino acid residues, is synthesised in the liver. For the first time, hepcidin was detected in urine and described by S.N. Park et al., Later, this peptide was also isolated from the plasma. A distinctive feature of the hepcidin molecule is the presence in it of disulfide bonds between two neighbouring molecules of the amino acid cysteine, which can determine the high reactivity of the molecule. With the development of systemic infection, hepcidin rises more than 100 times. However, as has been shown in recent years, the role of hepcidin is associated with clinical abnormalities in the parameters of iron metabolism, and some cases – with the development of anaemia.

In addition to hepatocytes, hepcidin mRNA is also expressed in the cells of the heart, lungs and placenta. Hepcidin regulates the intestinal absorption of iron, iron release by macrophages and placental passage of iron. Also, this hormone inhibits the release of iron from cells by binding and enhancing the degradation of the protein-iron exporter ferroportin

[11]. Hepcidin inhibits ferroportin, a specific carrier protein that carries iron to the interior of the cell, thereby impairing the absorption of iron in the small intestine. Another mechanism of action: hepcidin blocks the release of iron from macrophages, (locking) it inside the cell. Both these mechanisms lead to a violation of the homeostasis of iron, actually to iron deficiency and the development of anaemic syndrome, including in pregnant women. Analysis of literature data showed a limited number of studies about hepcidin in pregnant women. It was shown by Amat Bah et al. studies that the hepcidin concentration decreased by the 20<sup>th</sup> week of gestation, while the iron stores in the body of pregnant women decreased by the 30<sup>th</sup> gestation week [12]. The decrease in hepcidin level in pregnant women is suggested to be considered as a potential criterion for determining individual needs for intervention with iron preparations [13] [14]. At the same time, other data have been obtained that the concentration of hepcidin does not change depending on the trimester of pregnancy; there is also no correlation between hepcidin level and iron status [15].

Investigations of hepcidin level in pregnancy complications have been carried out. A study by M. Koenig et al., [16] showed an increase in serum hepcidin level in women with a high risk of pregnancy complications in comparison with healthy pregnant women. The hepcidin level was higher in pregnant women with pre-eclampsia than in the control group and positively correlated with the C-reactive protein. It is suggested that inflammation is a regulator of hepcidin production [11]. In inflammation, an increase of hepcidin level leads to a limitation of iron and a decrease in its availability for placental transfer.

Consequently, analysis of literature data has shown a limited number of studies of erythropoietin and hepcidin anaemia in pregnant women. Because, according to some authors, erythropoietin and hepcidin are in reciprocal relationships, their joint determination has a great interest AP.

In this regard, the purpose of our study was to study the content of erythropoietin and hepcidin in serum in pregnant women with iron deficiency anaemia and anaemia of chronic inflammation.

## Methods

We examined 98 pregnant women who were observed in LLP (Regional obstetric-gynaecological centre) in Karaganda. The including criteria for pregnant women in the study was the informed consent of the woman to participate in the study. Exclusion criteria were oncological diseases, HIV-infection, tuberculosis, severe somatic pathology, mental illness, drug addiction. The design of the study

was by the legislation of the Republic of Kazakhstan, international ethical norms and normative documents of research organisations, approved by the ethics committee of the Karaganda State Medical University.

The object of the study was blood. The level of erythropoietin and hepcidin in serum was determined by the method of enzyme immunoassay. A set of Vector Best production (Russian Federation) was used to determine erythropoietin; a Cloud Cloncorp set was used to determine hepcidin (Houston, USA). Also in the blood of patients, the level of haemoglobin and serum iron was determined spectrophotometrically using Sysmex KX-21N and A-15 analysers (BioSystems, Japan, Spain)

Pregnant women were divided into 3 groups: 22 women entered the group with the physiological course of pregnancy (1<sup>st</sup> group), 19 pregnant women with (iron deficiency anaemia) IDA (2<sup>nd</sup> group), 57 pregnant women with anaemia of chronic inflammation (ACI) (3<sup>rd</sup> group).

**Table 1: Characteristics of study groups**

Groups	Average age (years)	Average gestation (weeks)	Second trimester (persons)	Third trimester (persons)	Parity: The first pregnancy	Parity: The second pregnancy	Parity: The third and more pregnancy
Physiological pregnancy (22 persons)	33.13	31.81	2	20	10	6	6
IDA (19 persons)	30.38	32.77	1	18	3	6	10
ACI (57 persons)	29.51	36.11	2	55	12	29	16

The diagnosis of iron deficiency anaemia was established on the basis of the lowered norm of the level of iron in the blood (less than 8 mmol/l), the diagnosis of ACI was established on the basis of anamnestic data on the presence of chronic pyelonephritis, arterial hypertension and edema during a previous or existing pregnancy, normal serum iron level, the presence of proteinuria and/or leukocyturia.

The results were processed by statistical methods.

## Results

After determining the hepcidin and erythropoietin content of, a multidirectional change in these parameters in the serum of pregnant women of the 2<sup>nd</sup> and 3<sup>rd</sup> groups was observed. By the obtained data, 2 subgroups were singled out within the 2<sup>nd</sup> group and 4 subgroups within the 3<sup>rd</sup> group. The results of the study are presented in Table 1. It follows from the data in Table 1 that in pregnant women with IDA, a significant decrease in erythropoietin content is observed against a background of a decrease in haemoglobin and serum iron against a background of a clear trend towards an increase in hepcidin (subgroup 1). In pregnant women with IDA of

subgroup 2 erythropoietin level exceeds control indices, whereas the content of hepcidin was not different from the control.

In pregnant women with ACI of subgroup 1 the erythropoietin and haemoglobin content is significantly lower control indices, while the hepcidin level shows a distinct tendency to increase.

**Table 2: The level of haemoglobin, erythropoietin, hepcidin and iron in the investigated groups**

Group	Statistic value	Haemoglobin, g/l	Erythropoietin, miu/ml	Hepcidin, ng/ml	Iron, mmole/l
Control, N=22	Median	121.5	17.24	13.14	11.1
	Lower quartile	90	12.04	11.26	10.5
	Upper quartile	127.5	22.54	19.6	12.12
IDA, subgroup 1, n=12	Median	85.5*	7.20*	26.03	5.6*
	Lowerquartile	79.5	5.98	13.05	3.65
	Upperquartile	87.25	10.85	34.38	8.6
IDA, subgroup2, n=7	Median	88	23.19^	12	8.11*
	Lowerquartile	85.5	18.47	9.65	6
	Upperquartile	125.5	24.88	22.34	9.5
ACI Subgroup1, n=43	Median	87*	9.30*	24.9	10.5
	Lowerquartile	82.5	5.61	13.78	8.9
	Upperquartile	89	11.57	35.56	10.85
ACI Subgroup2, n=3	Median	89	7.64*	199.62*!	11.25
	Lowerquartile	79.5	6.81	192.35	10.42
	Upperquartile	108	8.15	250.75	11.52
ACI Subgroup3, n=7	Median	124#;	25.23*#;	12;	10.75
	Lowerquartile	118.5	23.51	10	10.1
	Upperquartile	129	26.43	21.39	12.1
ACI Subgroup4, n=4	Median	83.5?	14.60:?	89.9:?	10.05*:
	Lowerquartile	81	11.57	85.37	9.82
	Upperquartile	95.75	17.33	93.86	10.27

\*-reliability of differences with the control, p = 0.005; ^-reliability of differences in individuals with IDA between subgroups 1 and 3, p = 0.005; #-reliability of differences in persons with ACI between subgroups 1 and 4, p = 0.005; !-reliability of differences in persons with ACI between subgroups 1 and 2+3, p = 0.005; :-reliability of differences in individuals with ACI between clusters 2+3 and 4, p = 0.005; :-reliability of differences in individuals with ACI between clusters 2+3 and 5, p = 0.005; ?-the reliability of differences in individuals with ACI between clusters 4 and 5, p = 0.005.

Against a background of a significant decrease in the level of erythropoietin, a sharp, significant increase in hepcidin level was registered in pregnant women with ACI, included in the subgroup 2. There was a significant increase in erythropoietin, while serum hepcidin concentration did not differ from control, in pregnant women with ACI, included in subgroup the 3. There was a significant decrease in erythropoietin level of relative to the subgroups 3 and 2, with a significant increase in the content of hepcidin in pregnant women with ACI, included in the subgroup 4. Also in patients in this group, the serum iron level was reliably reduced. It should be noted that multidirectional changes in the concentrations of erythropoietin and hepcidin in the blood serum were recorded in a small proportion of patients with ACI.

## Discussion

It is known that an increased level of estrogen, accompanying pregnancy, causes a decrease in the production of erythropoietin. ACI is the result of inhibition of the synthesis of erythropoietin in the juxtaglomerular apparatus of the kidneys. Simultaneously, cytokines produced by macrophages in the presence of foci of chronic latent

inflammation, both in the organs of the female reproductive system and in other organs, in particular, the kidneys, contribute. An increase in the level of erythropoietin in the blood of pregnant women may reflect a high risk of preeclampsia [17].

As a result of the study, it was determined that the content of erythropoietin and hepcidin in pregnant women with anaemias of different genesis varies ambiguously. In the main group of pregnant women with IDA, the erythropoietin content rises, and the hepcidin level decreases. In pregnant women with ACI, on the contrary, the level of hepcidin increases, and in one subgroup it is significant. However, in pregnant women and with IDA and anaemia of chronic inflammation, there is a subgroup of women in whom erythropoietin is either comparable with hepcidin, or their changes are of opposite nature.

Thus, our data indicate ambiguous changes in the level of erythropoietin and hepcidin in pregnant women with anaemias of various origins. In all likelihood, there are still unaccounted factors affecting the content of these protein-regulators of iron metabolism, which require further definition and interpretation in anaemia of pregnant women.

## References

- Drakesmith H, Prentice AM, Hepcidin and the iron-infection axis. *Science*. 2012; 338:768–72. <https://doi.org/10.1126/science.1224577> PMID:23139325
- Prentice AM. Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism *Ann Nutr Metab*. 2017; 71(suppl 3):40–48. PMID:29268258
- Jelkmann W. Erythropoietin after a century of research: younger than ever. *European journal of haematology*. 2007; 78(3):183-205. <https://doi.org/10.1111/j.1600-0609.2007.00818.x> PMID:17253966
- Obeagu EI, Ezimah AC, Obeagu GU. Erythropoietin in the Anaemias of Pregnancy: A Review. *Int J Curr Res Chem Pharm Sci*. 2016; 3(3):10-8.
- Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, Taube DH, Bloom SR, Tam FW, Chapman R, Maxwell PH. Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. *haematologica*. 2010; 95(3):505-8. <https://doi.org/10.3324/haematol.2009.013136> PMID:19833632 PMID:PMC2833083
- Kowalska-Kańka A, Maciejewski T, Niemiec KT. The role and regulation of secretion of erythropoietin in pregnancy. *Medycyna wieku rozwojowego*. 2013; 3:270-5.
- McMullin MF, White R, Lappin T, Reeves J, MacKenzie G. Haemoglobin during pregnancy: relationship to erythropoietin and haematinic status. *European journal of haematology*. 2003; 71(1):44-50. <https://doi.org/10.1034/j.1600-0609.2003.00085.x> PMID:12801298
- Erdem A, Erdem M, Arslan M, Yazici G, Eskandari R, Himmetoglu Ö. The effect of maternal anemia and iron deficiency on fetal erythropoiesis: comparison between serum erythropoietin, hemoglobin and ferritin levels in mothers and newborns. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2002; 11(5):329-32. <https://doi.org/10.1080/jmf.11.5.329.332> PMID:12389675
- Ervasti M, Kotisaari S, Heinonen S, Punnonen K. Elevated serum erythropoietin concentration is associated with coordinated changes in red blood cell and reticulocyte indices of pregnant women at term. *Scandinavian journal of clinical and laboratory investigation*. 2008; 68(2):160-5. <https://doi.org/10.1080/00365510701550102> PMID:17852830
- Sienas L, Wong T, Collins R, Smith J. Contemporary uses of erythropoietin in pregnancy: a literature review. *Obstetrical & gynecological survey*. 2013; 68(8):594-602. <https://doi.org/10.1097/OGX.0b013e3182a2d51c> PMID:23921673
- Ganz T, Nemeth E. Iron sequestration and anemia of inflammation. *Semin Hematol*. 2009; 46:387–393. <https://doi.org/10.1053/j.seminhematol.2009.06.001> PMID:19786207 PMID:PMC2755591
- Bah A, Pasricha SR, Jallow MW, Sise EA, Wegmuller R, Armitage AE, Drakesmith H, Moore SE, Prentice AM. Serum Hepcidin Concentrations Decline during Pregnancy and May Identify Iron Deficiency: Analysis of a Longitudinal Pregnancy Cohort in The Gambia–3. *The Journal of nutrition*. 2017; 147(6):1131-7. <https://doi.org/10.3945/jn.116.245373> PMID:28424258 PMID:PMC5443464
- van Santen S, Kroot JJ, Zijdeveld G, Wiegerinck ET, Spaanderman ME, Swinkels DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. *Clinical chemistry and laboratory medicine*. 2013; 51(7):1395-401. <https://doi.org/10.1515/cclm-2012-0576> PMID:23241678
- Hedengran KK, Nelson D, Andersen MR, Stender S, Szecsi PB. Hepcidin levels are low during pregnancy and increase around delivery in women without iron deficiency—a prospective cohort study. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2016; 29(9):1506-8. PMID:26212583
- Simavli S, Derbent AU, Uysal S, Turhan NÖ. Hepcidin, iron status, and inflammation variables among healthy pregnant women in the Turkish population. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2014; 27(1):75-9. <https://doi.org/10.3109/14767058.2013.804054> PMID:23662610
- Koenig M, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. *Nutrients*. 2014; 6(8):3062-83. <https://doi.org/10.3390/nu6083062> PMID:25093277 PMID:PMC4145295
- Medvedev BI, Syundyukova EG, Sashenkov SL. Placental expression of erythropoietin in preeclampsia. *Rossiiskii vestnik akushera-ginekologa*. 2015; 15(1):4-8. <https://doi.org/10.17116/rosakush20151514-8>