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Pathogenesis of anemia in pregnant women with gestational diabetes mellitus

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ABSTRACT

The aim of the research was to establish the role of inflammation mediators and iron metabolism in the pathogenesis of various types of anemic syndrome in pregnant women with gestational diabetes mellitus (GDM).

Materials and methods. 32 pregnant patients with GDM were examined; 14 of them had iron deficiency anemia, 18 – anemia of chronic diseases. The enzyme-linked immunosorbent assay was used to determine the concentration of IL-6, hepcidin and a soluble receptor for transferrin in the blood serum of pregnant women, the concentrations of C-reactive protein and transferrin were determined with the method of turbidimetry.

Results. It was shown that women with GDM had higher IL-6 level compared to healthy pregnant women, and the concentration of IL-6 did not depend on the type of anemic syndrome. The C-reactive protein concentration was higher in patients with GDM and anemia of chronic diseases than in healthy pregnant women or in pregnant women with iron deficiency anemia. An analysis of iron metabolism markers in pregnant women with GDM established that patients with anemia of chronic diseases had higher hepcidin levels than women with iron deficiency anemia or healthy pregnant women.

Conclusions. We established the heterogeneity of the anemic syndrome in pregnancy complicated by GDM. It was confirmed that GDM was accompanied by subclinical inflammation, which was more pronounced in anemia of chronic diseases. The research showed that the mechanism of development of anemia of chronic diseases involving the hepcidin protein was also realized in GDM, characterized by subclinical inflammation. The results indicate the importance of establishing the type of the anemic syndrome in pregnant women with GDM for effective therapeutic follow-up.

Key words: pregnancy, anemia of chronic diseases, gestational diabetes mellitus, inflammation, hepcidin.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 3431, 2013).

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Патогенез анемического синдрома у беременных с гестационным сахарным диабетом

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РЕЗЮМЕ

Цель. Установить роль медиаторов воспаления и метаболизма железа в патогенезе различных видов анемического синдрома у беременных с гестационным сахарным диабетом (ГСД).

Материалы и методы. Проведено обследование 32 беременных с ГСД, из которых 14 пациенток имели железодефицитную анемию, а 18 – анемию хронических заболеваний. В сыворотке крови беременных методом иммуноферментного анализа определяли концентрацию интерлейкина 6, гепсидина, растворимого рецептора к трансферрину, методом турбидиметрии – концентрацию С-реактивного белка и трансферрина.

Результаты. У беременных с ГСД концентрация интерлейкина 6 повышена в сравнении с его уровнем у здоровых беременных и не зависит от вида анемического синдрома. У беременных с ГСД, имевших анемию хронических заболеваний, концентрация в крови С-реактивного белка превышала таковую у здоровых беременных и беременных с железодефицитной анемией. Анализ содержания в крови у беременных с ГСД маркеров метаболизма железа показал, что у женщин с анемией хронических заболеваний концентрация гепсидина значительно выше, чем у пациенток с железодефицитной анемией и здоровых беременных.

Заключение. Установлена гетерогенность анемического синдрома при беременности, отягощенной ГСД: истинную железодефицитную анемию имели менее половины пациенток, в то время как у большей части женщин анемия была связана с внутриклеточной блокадой железа. Подтверждено, что ГСД сопровождается развитием субклинического воспаления, более выраженного при анемии хронических заболеваний. Показано, что механизм развития анемии хронических заболеваний с участием белка гепсидина реализуется и при ГСД, характеризующимся субклиническим воспалением. Полученные результаты указывают на важность установления вида анемического синдрома у беременных с ГСД с целью эффективного терапевтического сопровождения пациенток.

Ключевые слова: беременность, анемия хронических заболеваний, гестационный сахарный диабет, воспаление, гепсидин.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с проведенным исследованием и публикацией настоящей статьи.

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INTRODUCTION

Carbohydrate metabolism disorder and anemic syndrome are some of the most common extragenital diseases in pregnant women. According to the World Health Organization (2014), in most cases, anemia in pregnant women is iron deficiency anemia, and its frequency in developed countries reaches up to 20–25%, in Russia – up to 35–43%. However, due to glucose toxicity and lipotoxicity, anemia of chronic diseases resistant to iron therapy is very likely to develop in pregnant women with carbohydrate metabolism disorders. High prevalence of the anemic syndrome together with its heterogeneity (in the presence of a concomitant pathology) in pregnant women and possible complications (increased perinatal mortality, fetal growth retardation, intrauterine hypoxia, neonatal asphyxia, weak labor, poor tolerance of blood loss, etc.) make both studying the anemia pathogenesis and improving the methods of its laboratory diagnosis essensial [1].

The aim of the study was to establish the role of inflammation mediators and iron metabolism in the pathogenesis of various types of the anemic syndrome in pregnant women with gestational diabetes mellitus.

MATERIALS AND METHODS

The study included 32 pregnant women with gestational diabetes mellitus (GDM) followed up by an endocrinologist at Regional Perinatal Center (Tomsk). Gestational diabetes mellitus was diagnosed in the first trimester of pregnancy during the first biochemical screening according to the diagnostic criteria in compliance with the Russian National Consensus Statement on gestational diabetes mellitus: diagnosis, treatment and postnatal care, 2012. In all patients, GDM was treated with diet therapy, without the use of insulin preparations. The anemic syndrome was diagnosed and specified following complex analysis of laboratory data. The hematology profile (red blood cells, reticulocytes, hemoglobin) (using a 5-diff hematology analyzer Sysmex xs-1000i, Sysmex, Japan) and blood serum chemistry (serum iron, total iron binding capacity, serum ferritin) (using a Cobas analyzer with 311, Roche, Germany) were studied. A decrease in hemoglobin concentration lower than 110 g/l was considered as the anemic syndrome in pregnant women. Iron deficiency anemia was verified in case of a decrease in blood levels of serum iron and ferritin, in combination with increased total iron binding capacity. Anemia of chronic diseases was established in case of reduced total iron binding capacity and serum iron concentration, but normal or increased ferritin concentration. All pregnant women were divided into two groups: iron deficiency anemia (IDA) – 14 patients, and anemia of chronic diseases (ACD) – 18 patients.

At the time of the study, all patients were in the second trimester of pregnancy, aged 23 – 44 years. The control group consisted of 12 healthy pregnant women of comparable age. Exclusion criteria were the use of iron preparations, acute infections or exacerbation of chronic infections, purulent necrotic diseases, allergic diseases (bronchial asthma, atopic dermatitis, etc.) in the medical history or at the time of the screening, nephritis of any etiology, psoriasis, and refusal to participate in the study. The study met ethical standards; all individuals participating in the study signed an informed consent.

The study material was venous blood serum taken in the morning before ingestion from the ulnar vein in an amount of 10 ml into a BD Vacutainer vacuum tube with a coagulation activator with silica particles (Becton Dickinson, USA). The concentrations of IL-6 (pg/ml) (Vector-Best, Russia), hepcidin (ng/ml) (MyBioSource, USA) and soluble transferrin receptor (sTfR) (nmol/l) (R&D Systems, USA) were determined by the serum-linked immunosorbent assay in blood serum according to the instructions of reagent kit manufacturers. The results were read using an automatic photometer for Sunrise microplates (Tecan, Austria) at a wavelength of 450 nm. The concentration of the studied markers was determined by a standard calibration curve. The concentrations of C-reactive protein (CRP) (mg/l) (at a wavelength of 552 nm) and transferrin (g/l) (at a wavelength of 570 nm) were determined in the blood serum by the turbidimetric method using a Cobas c311 analyzer (Roche, Germany).

Statistical processing of the obtained data was performed using the SPSS Statistics 18 software package. The distribution normality was checked using the Shapiro – Wilk criterion. The threshold level of statistical significance of differences was 0.05.

The data obtained did not obey the normal distribution law and were presented as the median and interquartile range (*Me*, Q_{25}–Q_{75}). The significance of differences between independent comparison groups was established using the Kruskal – Wallis criteria with the Bonferoni correction for the three study groups, and using the Mann – Whitney test for two groups. The correlation between the studied parameters was evaluated using the Spearman's test for nonparametric data.

RESULTS

It was found that the concentration of IL-6 increased in pregnant women with GDM, ($p < 0.05$) compared to healthy pregnant women and did not depend on the type of the anemic syndrome. CRP level was also higher in patients with GDM and ACD compared to patients with IDA and healthy pregnant women ($p < 0.05$ for both cases) (Table 1).

The analysis of iron metabolism markers in women with GDM showed that patients with ACD had higher hepcidin concentrations, than those with IDA ($p < 0.05$) and healthy pregnant women ($p < 0.05$). An average positive linear relationship was found between the concentrations of hepcidin and CRP ($r = 0.61$; $p < 0.05$) in the blood serum of patients with gestational diabetes mellitus and anemia of chronic diseases. There were no differences in transferrin and sTfR concentrations in IDA and ACD patients ($p > 0.05$ in all cases) (Table).

Table

The concentration of inflammatory and iron metabolism markers in the serum of pregnant women with gestational diabetes mellitus, taking into account the type of anemia, *Me* (Q_{25}–Q_{75})

Parameter	Pregnant women without gestational diabetes mellitus and anemia ($n = 12$)	Pregnant women with gestational diabetes mellitus	
		Pregnant women with iron deficiency anemia ($n = 14$)	Pregnant women with anemia of chronic disease ($n = 18$)
IL-6 (pg/ml)	0.5 (0–1.0)	2.8 (2.6–2.8)*	2.7 (1.8–3.0)*
C-reactive protein (mg/l)	1.33 (1.0–1.65)	2.82 (1.85–3.29)	8.79 (6.32–10.12) * **
Hepcidin (ng/ml)	5.55 (0–11.1)	7.1 (3.4–11.7)	12.2 (11.6–14.6)* **
sTfR (nmol/l)	20.15 (5.94–34.36)	34.79 (23.92–38.85)	38.41 (24.5–42.47)
Transferrin (g/l)	–	4.03 (3.65–4.39)	4.05 (4.05–4.15)

* the differences are significant against similar indexes in pregnant women without gestational diabetes mellitus and anemia ($p < 0.05$); ** against pregnant women with iron deficiency anemia.

However, we found a strong positive linear relationship between the increasing concentrations of transferrin and its soluble receptor ($r = 0.84$; $p < 0.05$) in the blood serum of patients with GDM and IDA, which reflects the classical concept of IDA pathogenesis.

DISCUSSION

Already at the stage of patients' stratification into clinical examination groups, the study showed that in almost half of cases anemia in pregnant women with GDM was not iron deficiency anemia and, therefore, could not be treated by iron preparations. The heterogeneity of the structure of the anemic syndrome in individuals with type 1 diabetes mellitus (both in pregnant and non-pregnant patients with type 1 diabetes mellitus) has been confirmed by other studies [2, 3]. Thus, it is necessary to clearly differentiate anemia of chronic diseases and iron deficiency anemia in pregnant women with impaired carbohydrate metabolism.

It was established that in anemia of chronic diseases, the key role in the activation of hepcidin synthesis belongs to the group of proinflammatory cytokines – IL-1, IL-6, TNFα [4], and especially to IL-6 [5]. The main function of hepcidin, in turn, is to block the action of the iron carrier protein, ferroportin, as a result of which iron is disrupted from macrophages, enterocytes, placenta, and other cells, leading to hypoferremia [6]. Thus, the pathogenesis of anemia of chronic diseases is associated with an excess of IL-6 production, activation of hepcidin synthesis and a decrease in the availability of iron for erythropoiesis. This study, as well as our previous works [7, 8], indicated the presence of an inflammatory process in GDM, since the concentration of IL-6

in the blood serum of women with GDM was higher than during physiological pregnancy. It should be noted that the concentration of IL-6 in pregnant women with GDM had comparable values for various types of the anemic syndrome. Moreover, a significant increase in hepcidin concentration was observed only in pregnant women with anemia of chronic diseases. This indicates the presence of additional factors that stimulate hepcidin production and contribute to the development of anemia of chronic diseases in GDM. The most sensitive clinical and laboratory indicator of inflammation is C-reactive protein. The highest concentration of C-reactive protein was recorded in the blood serum of pregnant women with GDM who had anemia of chronic diseases, which indicates a greater activation of inflammation in these patients, compared with pregnant women suffering from iron deficiency anemia. Therefore, the mechanism of development of anemia of chronic diseases involving hepcidin, IL-6 and C-reactive protein is also realized in subclinical inflammation accompanying gestational diabetes mellitus.

Transferrin and sTfR are considered as differential diagnostic markers of IDA and ACD. It is stated that patients with anemia of chronic diseases have normal or reduced concentrations of transferrin and sTfR in the blood serum, while in patients with iron deficiency anemia the concentrations of these markers increase [9, 10]. However, the content of transferrin and sTfR in the blood reflects both the amount of iron deposited in the body and the activity of erythropoiesis [11, 12], which increases by the end of the first trimester of pregnancy. Thus, the informative value of these tests after 12 weeks of pregnancy is doubtful.

This study confirmed the limitation of using transferrin and its soluble receptor as indicators of ACD verification in pregnant women with GDM.

CONCLUSION

Gestational diabetes mellitus is accompanied by subclinical inflammation, which is more pronounced in anemia of chronic diseases, as opposed to iron deficiency anemia. The mechanism of development of anemia of chronic diseases in GDM involves the hepcidin protein. The heterogeneity of the anemic syndrome in pregnancy complicated by GDM was established: less than half of patients had true iron deficiency anemia, while in most women anemia was associated with intracellular iron blockade. The results indicate the importance of correct differential diagnosis of the anemic syndrome in pregnant women with GDM.

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Authors contribution

Zima A.P. – conception and design, final approval of the manuscript for publication. Prokhorenko T.S. – carrying out of experimental work, analysis and interpretation of data. Saprina T.V. – analysis and interpretation of data. Musina N.N. – carrying out of experimental work, processing of data. Novitsky V.V. – substantiation and critical revision of the manuscript for important intellectual content. Baykov A.N. – interpretation of the results, drafting of the manuscript.

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