ASSESSMENT OF BLOOD AMINO ACID AND POLYAMINE LEVELS IN PLACENTA-ASSOCIATED PREGNANCY COMPLICATIONS

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The features of polyamine and amino acid metabolism play a key role in the cellular processes, and the search for their role as prognostic and diagnostic (assessment of fetal condition severity) markers in obstetrics can contribute to improvement of perinatal outcomes in fetal growth restriction (FGR) syndrome, both isolated and combined with early onset preeclampsia (PE). The study was aimed to determine the features of polyamine and amino acid levels associated with placenta-associated pregnancy complications. Liquid chromatography coupled with mass spectrometry was used to determine blood levels of polyamines and amino acids in 156 pregnant women divided into the following groups: with FGR — 48 pregnant women, with early onset PE — 56 pregnant women, control group — 52 somatically healthy women having no pregnancy complications. As a result, we managed to distinguish significant differences in these metabolites, depending on the obstetric complication (PE or FGR), and to determine correlations of those with a number of clinical data. We revealed a strong negative correlation between the increasing fetal condition decompensation in FGR and the length of the newborn's hospital stay for the PE and FGR groups, as well as between the levels of 1,7-diaminoheptane polyamine (r = –0.78, CI = –0.92 – –0.37, p = 0.002; r = –0.76, CI = –0.95 – 0.23, p = 0.003) and proline amino acid and the increasing fetal condition decompensation in FGR (r = –0.56, CI = –0.86 – –0.034, p = 0.03). Considering the diversity and complexity of metabolic pathways responsible for adaptation in the context of hypoxic damage, the results obtained suggest that regulation of amino acids and polyamines is coordinated. Metabolic pathways of low molecular weight antioxidants, proline and polyamines, are associated with clinical pregnancy outcomes in FGR and early-onset PE.

Keywords: intrauterine growth restriction, fetal growth restriction, preeclampsia, metabolomics, newborn, mass spectrometry, biomarkers, polyamines, aminoacids

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ИЗУЧЕНИЕ УРОВНЯ АМИНОКИСЛОТ И ПОЛИАМИНОВ КРОВИ ПРИ АССОЦИИРОВАННЫХ С ПЛАЦЕНТОЙ ОСЛОЖНЕНИЯХ БЕРЕМЕННОСТИ

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Особенности метаболизма полиаминов и аминокислот играют ключевую роль в клеточных процессах, а поиск их роли в качестве прогностических и диагностических (оценка тяжести состояния плода) маркеров в акушерстве может способствовать улучшению перинатальных исходов при синдроме задержки роста плода (ЗРП), как изолированном, так и сочетанном с ранней преэклампсией (ПЭ). Целью исследования было определить особенности уровней полиаминов и аминокислот, сопряженных с плацента-ассоциированными осложнениями беременности. С помощью жидкостной хроматографии с масс-спектрометрическим детектированием были определены уровни полиаминов и аминокислот в крови 156 беременных женщин, разделенных на группы: с ЗРП — 48 беременных, с ранней ПЭ — 56 беременных, в контрольной группе — 52 соматически здоровых женщины с беременностью без осложнений. В результате удалось выделить значимые отличия данных метаболитов, в зависимости от акушерского осложнения (ПЭ или ЗРП) и установить их корреляционную зависимость с рядом клинических данных. Обнаружена сильная обратная корреляционная связь с нарастанием декомпенсации состояния плода при ЗРП и числом койко-дней, проведенных в стационаре новорожденным для групп ПЭ и ЗРП и уровнем полиамина 1,7-диаминогептан (r = –0,78, CI = –0,92 – –0,37, p = 0,002; r = –0,76, CI = –0,95 – 0,23, p = 0,003) и аминокислотой пролин и нарастанием декомпенсации состояния плода при ЗРП (r = –0,56, CI = –0,86 – –0,034, p = 0,03). Учитывая многообразие и сложность метаболических путей, ответственных за адаптацию в условиях гипоксического поражения, на основании полученных результатов, можно предположить, что регуляция аминокислот и полиаминов является координированной. Пути метаболизма низкомолекулярных антиоксидантов — пролина и полиаминов ассоциированы с клиническими исходами беременности при ЗРП и ранней ПЭ.

Ключевые слова: задержка внутриутробного развития, ограничение роста плода, преэклампсия, метаболомика, новорожденный, масс-спектрометрия, биомаркеры, полиамины, аминокислоты

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Polyamines are polymeric molecules containing several amino groups. These are involved in the broad spectrum of biochemical processes, such as generation of membrane potential, DNA and RNA stabilization, enzyme activation, and ion transport, as well as in regulation of gene expression, protein synthesis; polyamines exert antitoxic activity, have an effect on the body's immune system [1–2].

Since polyamines are derived from amino acids [3], these can be considered an essential component of amino acid metabolism and cell regulation [4]. Polyamine levels can be regulated by changing bioavailability and the concentrations of amino acids essential for synthesis of polyamines [5]. For polyamines to perform their functions in various cells and tissues, these must penetrate cell membranes. This process involves the transport systems, in which amino acids ensure transfer of those through the membrane. Therefore, the link between polyamines and amino acids covers not only polyamine synthesis, but also the effects of polyamines on the cellular processes, including protein regulation and interaction with cell membranes. This is a complex area of research that is of great importance for understanding the cell physiology and biochemistry.

Medical use of polyamines is still evolving, however, there are already several fields, in which these molecules can be used as biomarkers for diagnosis of various disorders: cancer [6, 7], neurodegenerative disorders [8], infectious diseases, and autoimmune disorders [9].

In obstetrics, polyamines are associated with various aspects of pregnancy and childbirth [10], including cell growth and differentiation associated with the development of fetal organs and systems [11–13]. The link between amino acids and polyamines is of high clinical significance. Such an essential amino acid, as arginine, is a precursor of polyamines. Arginine is converted to spermidine polyamine and spermine through the series of biochemical reactions. Cadaverine also can be synthesized from arginine and can take part in regulation of a number of cellular processes. Low levels of arginine, lysine, and aspartic acid can play an important role in the processes resulting in fetal growth restriction (FGR). Arginine, lysine, and aspartic acid are not only involved in protein synthesis, but are important for many biochemical pathways and body functions [14]. Arginine is a precursor of nitric oxide (NO) playing an important role in regulation of vascular function. NO dilates blood vessels and improves blood flow, and this is the key factor ensuring normal growth and development of the fetus. Low arginine levels can decrease NO production and compromise vascular response, thereby limiting fetal nutrient supply [15]. Lysine deficiency can result in the decreased synthesis of certain proteins and affect fetal growth. Furthermore, lysine is involved in the fat and carbohydrate metabolism, and low levels of these amino acids can compromise fetal energy supply. Aspartic acid can affect metabolic pathways of other amino acids, including those associated with fetal growth and development.

Amino acids play a role in regulation of blood pressure and vascular function. The research shows that alterations of maternal amino acid profile can be associated with the development of preeclampsia (PE). For example, high levels of glutamic and aspartic acids are associated with the risk of PE [16] .

Determination of amino acid and polyamine levels in mother's body and the fetus can provide important information about the risk of FGR and PE. The research continues, and better understanding of the relationships between amino acids, polyamines, and these obstetric conditions can lead to the development of more effective methods for diagnosis and monitoring of pregnancy, as well as to improvement of treatment and prevention strategies.

The study was aimed to determine the features of polyamine and amino acid levels associated with the placenta-associated pregnancy complications, including FGR (both isolated and combined with early-onset PE), as well as to reveal clinical and laboratory parallels in FGR and PE based on the correlation analysis of the relationship between the levels of important polyamines and amino acids and such clinical parameters, as FGR and PE severity, length of hospital stay in the newborns born to mothers of the FGR and PE groups.

METHODS

A total of 156 pregnant women, who were admitted to and gave birth in the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, were included in the study: PE and FGR groups consisted of 56 and 48 patients with the confirmed appropriate diagnoses; the control group included 52 somatically healthy women with no pregnancy complications. Inclusion criteria: pregnant women's age 18–35 years, 24–40 weeks of singleton pregnancy, early-onset preeclampsia and fetal growth restriction. Exclusion criteria: Rh isoimmunization and ABO incompatibility, chromosomal abnormalities, genetic mutations and congenital malformations in the fetus, severe extragenital disorder, chronic kidney disease, large uterine fibroid, acute infectious disease in the mother. Venous blood was collected for analysis.

Polyamine analysis method

The optimized procedure of blood sample preparation for assessment of polyamine levels includes the following phases: add 1200 μL of methanol to 400 μL of blood plasma, stir for 5 min, centrifuge for 10 min at 13,000 g (Eppendorf MiniSpin centrifuge, Germany), collect 1000 μL of supernatant, dry in nitrogen flow at the temperature of 50 °C, add 600 μL of the 10 mg/mL dansyl chloride solution in the acetonitrile/carbonate buffer with pH = 9.7 (50/50 v/v), stir for 1 min, centrifuge for 1 min at 13,000 g, incubate at 60 °C for 90 min, centrifuge for 1 min at 13,000 g, add 1000 μL of ethyl acetate, stir for 10 min, centrifuge for 10 min at 13,000 g, collect 1000 μL of the top layer, add 1000 μL of ethyl acetate, stir for 10 min, centrifuge for 10 min at 13,000 g; collect 1000 μL of the top layer and combine with the previously collected portion, dry in nitrogen flow at the temperature of 50 °C, add 200 μL of acetonitrile, stir for 5 min, centrifuge for 10 min at 13,000 g; take 170 μL for further analysis.

The analysis of polyamines and amino acids was conducted by liquid chromatography–mass spectrometry (LC–MS) in the system consisting of the ABSciex QTrap 5500 triple quadrupole mass spectrometer (ABSciex; Canada) coupled to electrospray ionization and the Agilent 1260 Infinity liquid chromatography system (Agilent; USA). The Agilent Zorbax Eclipse Plus C18 column (50 — 3 mm, 1.8 μm; Agilent, USA) was used for separation of the sample. To analyze organic acids, we introduced 20 μL of the sample and used the 0.1% aqueous formic acid solution as eluent A; eluent B was the 0.1% acetonitrile formic acid solution. The flow rate was 650 μL/min, and the column temperature of 30 °C was maintained. The mobile phase composition changed during the analysis in the following way: 0–0.3 min — 20% of eluent B, by 5.3 min the volume fraction of eluent B increased to 95%, remained the same by 8.3 min, and returned to the value of 20% within 0.1 min. The mass spectrometer settings were as follows: peripheral gas pressure — 1.4 bar, nebulizer gas pressure — 3.4 bar, source temperature — 500 °C, capillary voltage — 4500 V.

Table 1. Clinical characteristics of the studied groups

Parameter	Descriptive statistics if the parameter			Significance of differences in the parameters for pairwise comparison of the group (*p*-value)		
	PE (*n* = 56)	FGR (*n* = 48)	Normal (*n* = 52)	PE – Normal	FGR – Normal	PE – FGR
Age	32 ± 5	32 ± 3	31 ± 4	0.654	0.212	0.146
BMI (pregravid)	27 ± 5	20 ± 5	25 ± 3	0.745	0.005	0.003
Gestational age at delivery	35 ± 3	37 ± 2	40 ± 1	< 0.001	< 0.001	0.435
Birth length	45.8 ± 4.5	45.1 ± 2.1	52.1 ± 2.2	0.001	< 0.001	0.378
Birth weight	2132 ± 846.6	1969.7 ± 501.0	3403.7 ± 395.7	< 0.001	<0.001	0.401
Apgar score 1	7 (7; 8)	8 (8; 8)	8 (8; 8)	< 0.001	0.357	0.06
Apgar score 5	8 (8; 9)	9 (8; 9)	9 (9; 9)	0.02	0.033	0.031

In this study, the following nine polyamines were analyzed: putrescine, ethylenediamine, 1,3-diaminopropane, cadaverine, 1,7-diaminoheptane, cadaverine, N-acetylputrescine, N1-acetylspermine, spermidine; along with 43 amino acids: 1-methylhistidine, 3-aminoisobutyric acid, 3-methylhistidine, argininosuccinic acid, beta-alanine, creatinine, 5-hydroxylysine, homocystine, ethanolamine, gamma-aminobutyric acid, 2-aminoadipic acid, 2-aminobutyric acid, alanine, anserine, arginine, asparagine, aspartic acid, carnosine, citrulline, cystathionine, cystine, glutamic acid, glutamine, glycine, histidine, homocitrulline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, O-phosphorylethanolamine, sarcosine, taurine, trans-4-hydroxyproline.

Statistical analysis

Statistical processing of the experimental data obtained was performed using the R scripts.

Statistical analysis involved the use of the nonparametric Mann–Whitney U test. Quantitative data were described using the median (Me) and Q_1 and Q_3 quartiles in the Me (Q_1; Q_3) format, along with the mean (M) and standard deviation (SD) in the M ± SD format. The threshold significance level p was considered to be equal to 0.05. When p-value was below 0.001, it was provided in the $p < 0.001$ format.

The search for clinical and laboratory parallels in FGR and PE was performed using the Spearman rank correlation test for the polyamine and amino acid levels and clinical indicators. The correlation was considered significant at $p < 0.05$.

The logistic regression models were constructed to assess the possibility of classifying patients into groups based on the studied parameters. From all the models constructed we chose four with the largest area under the ROC curve (AUC). Quality of the models constructed was determined by plotting the ROC curve and calculating sensitivity and specificity.

RESULTS

Pregnant patients were included in the case-control study, as they contacted the Center after being diagnosed with FGR or PE. At the time of inclusion in the study and blood collection, patients of the PE group had moderate PE with stable blood pressure, negligible proteinuria with normal 24-hour diuresis, no fetal disorder based on the Doppler data (fetoplacental blood flow and fetal distress); patients of the FGR group (3rd percentile and below based on fetometry data) showed no Doppler signs of fetal distress. All the patients had no regular labor contractions or threatened preterm labor. At the time of delivery, which happened on average three weeks after blood collection, one-third of the patients had the diagnosis of severe preeclampsia, and critical disturbances of fetoplacental circulation were found in more than a half of the patients, which was the indication for surgical delivery through cesarean section. In patients of the control group, blood collection was performed at the gestational age similar to that of patients with PE and FGR. Later their course of pregnancy and delivery were monitored and considered to be uncomplicated.

Given the fact, that advanced reproductive age and obesity could become serious confounding factors when assessing blood levels of amino acids and polyamines, the groups were formed so that the age and body weight of patients with PE were matched to that of the control group. Body mass index (BMI) of pregnant women of the FGR group was significantly lower, that that of women of the Normal and Preeclampsia groups ($p = 0.005$ and $p = 0.003$, respectively).

The results of assessing clinical parameters of the studied groups are provided in Table 1.

Due to the need for accelerated delivery for obstetric reasons resulting from the increasing PE severity and progressive intrauterine fetal hypoxia in FGR, the gestational age at delivery in these groups was significantly different from that in the control group ($p < 0.001$). In cases of PE and FGR, the average weight of newborns was 2132.0 g and 1969.7 g.

It should be noted, that the newborns' Apgar scores in the group with PE were significantly lower in the first minute of life ($p < 0.001$), while in the fifth minute there were significant differences in Apgar scores between all groups, which was likely to be associated with impaired adaptation in the early neonatal period in the newborns from the PE and FGR groups, who experienced chronic hypoxia for a long time.

Due to the important role of amino acid in polyamine synthesis and their function in cell metabolism, assessment of amino acid levels in patients of the studied groups was conducted in the first phase of laboratory analysis.

Data analysis made it possible to reveal the decrease in the levels of a number of amino acids relative to the control group in cases of PE and FGR: patients with FGR showed a significant decrease in the levels of alanine ($p = 0.0136$), ornithine ($p = 0.045$), proline ($p = 0.0044$) , while patients with PE showed a significant decrease in aspartic acid levels ($p = 0.0422$) (Fig. 1). Low levels (relative units) of arginine, 0.0163 (0.0139, 0.0182), and lysine, 0.0524 (0.0492, 0.056), associated with FGR, can play an important role in FGR syndrome.

Then we determined serum polyamine levels in patients of the studied groups. In the group of patients with PE, we revealed significant differences from the control group for putrescine ($p = 0.0423$) and spermidine ($p = 0.022$); in the FGR group significant differences from the control group were reported for cadaverine ($p = 0.0282$). When comparing the data of the PE

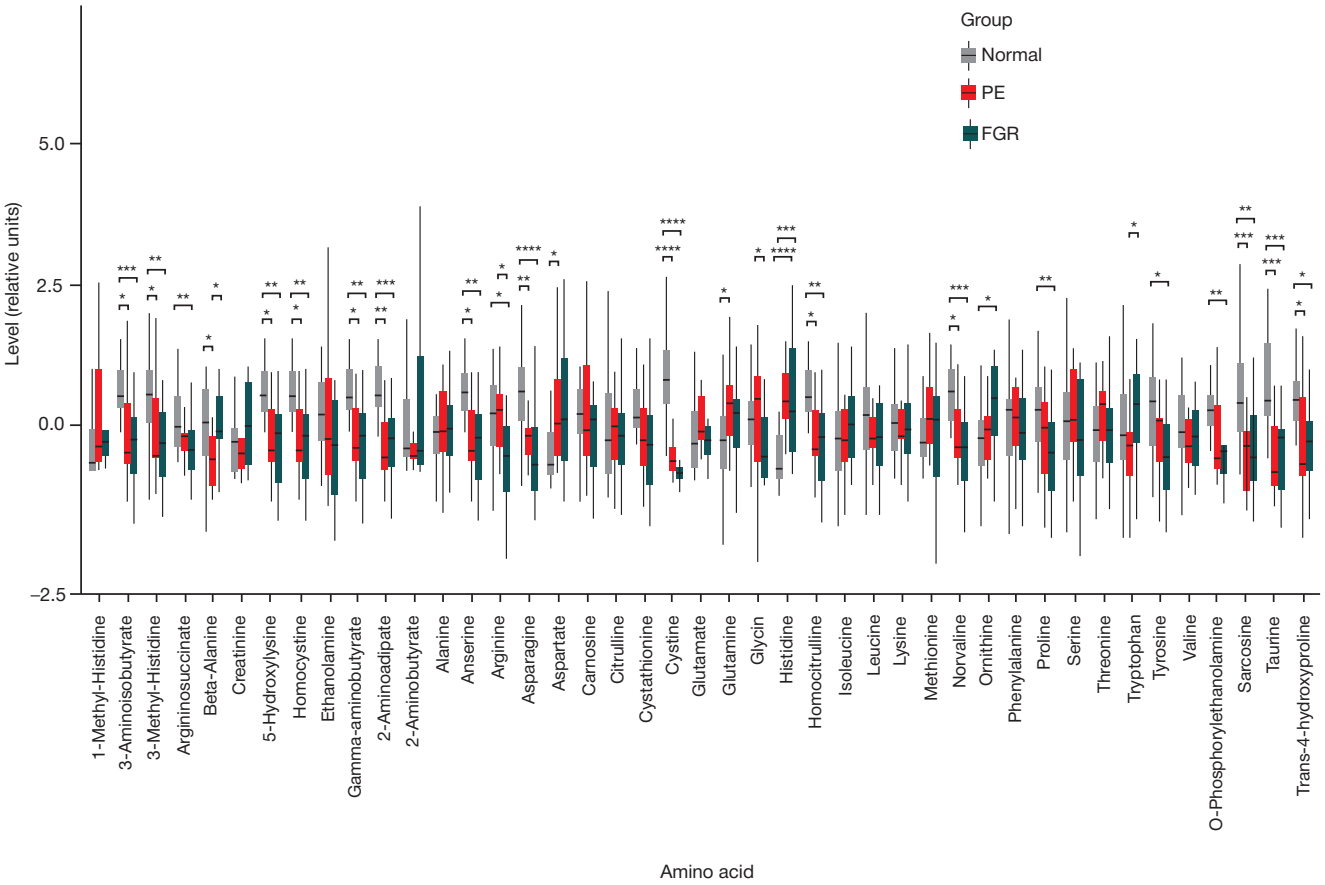

Fig. 1. Comparison of plasma amino acid profiles in patients of the groups Normal, PE, and FGR. The first and third quartiles are the margins of the box, while the line in the middle of the box is the median; the ends of whiskers are the difference between the first quartile and the 1.5 interquartile range, the sum of the third quartile and the 1.5 interquartile range

and FGR groups, significant differences were reported for two polyamines: putrescine (p = 0.0039) and 1,7-diaminoheptane (p = 0.0091) (Fig. 2).

In human body, putrescine is derived from L-ornithine, which involves the ornithine decarboxylase (ODC) enzyme. Spermidine is synthesized from putrescine, which involves two enzymes: spermidine synthase and spermine synthase. These enzymes are the most short-living human enzymes (half-life 5–10 min), which indicates that these are directly involved in protein biosynthesis. Activity of these enzymes can be indirectly estimated based on the spermidine to putrescine ratio (spermidine synthase activity) and spermine to spermidine ratio (spermine synthase activity) (Fig. 3).

The spermidine to putrescine ratio was –0.33 (–0.43; 0.05) in the Normal group of patients, –0.21 (–0.29; 0.25) in the group of patients with PE, and –0.34 (–0.44; –0.13) in the group of patients with FGR; the spermine to spermidine ratios of these groups were 0.07 (–0.38; 0.57); –0.48 (–0.64; –0.08); –0.33 (–0.65; 0.52), respectively.

Polyamines are deeply involved in the cellular mechanisms underlying nonspecific stress responses (polyamine stress response). In case of damaging effect on the body, for example when there is nervous tissue ischemia/hypoxia, ODC is activated in the brain, with subsequent increase in polyamine content and triggering of activation of the early response genes (*c-myc*, *c-fos*, etc.) [17].

Considering biological significance of the findings, the search for clinical and laboratory parallels in FGR and PE was of particular interest. For that we performed correlation analysis of the relationship between the levels of important polyamines and amino acids and such clinical indicators, as FGR and PE course severity, length of hospital stay in the newborns born to mothers of the FGR and PE groups (Table 2).

Table 2 provides a significant correlation between clinical parameters and plasma levels of amino acids. Plasma levels of trans-4-hydroxyproline showed a significant negative correlation with the fetal condition decompensation in FGR, along with the significant positive correlation with the child's birth weight. Similarly, proline levels showed a significant positive correlation with the child's weight and a significant negative correlation with the fetal condition decompensation in FGR. We also determined that glutamine levels showed a positive correlation with PE severity and a negative correlation with the aspartic acid levels and the length of hospital stay.

Similar analysis was performed for polyamines (Table 2). As for FGR, we revealed a correlation between the severity of this complication and the levels of 1,7-diaminoheptane and cadaverine. Putrescine levels were correlated to PE severity. The length of the newborn's hospital stay in the PE and FGR groups was correlated to the 1,7-diaminoheptane levels.

Of interest are the correlations with proline and 1,7-diaminoheptane revealed. These metabolites show moderate and strong correlations with the clinical data that characterize obstetric outcomes: the child's birth weight and the length of hospital stay, respectively, which makes it possible to consider these as prognostic markers for dynamic assessment of the increasing severity of FGR and early-onset PE.

The mathematical models were constructed in order to ensure primary assessment of the prognostic and diagnostic (assessment of fetal condition severity) capabilities of the above metabolites in terms of assessing the severity of the PE and FGR course and optimization of obstetric tactics for

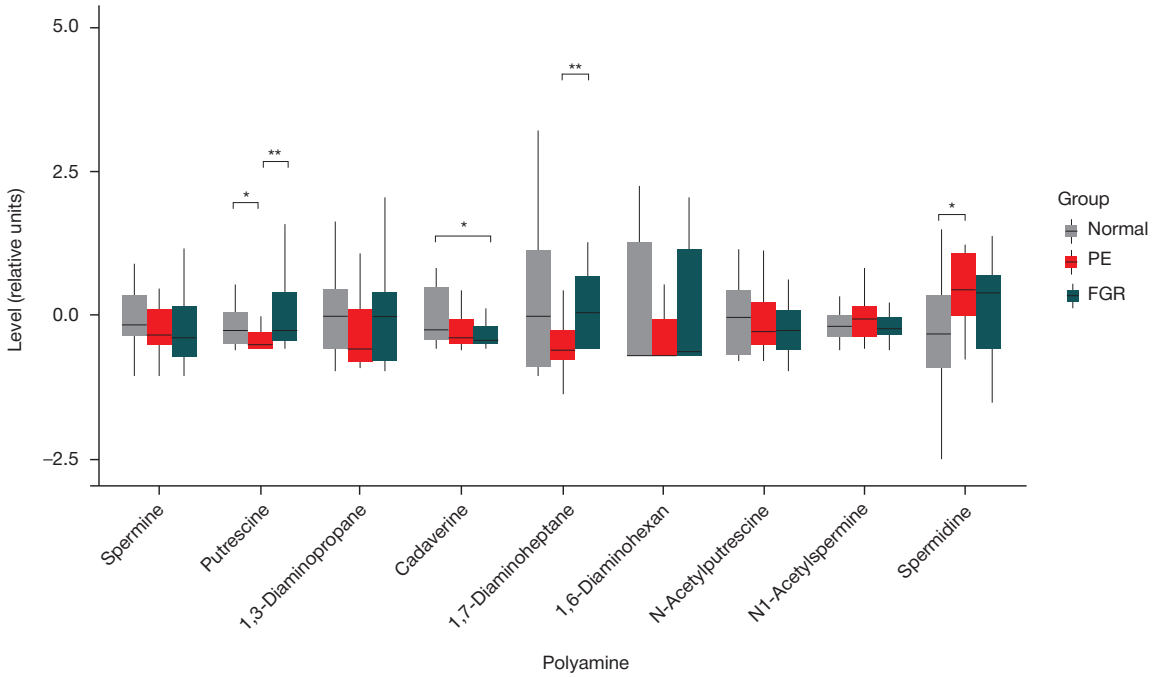

Fig. 2. Comparison of plasma polyamine profiles in patients of the groups Normal, PE, and FGR. The first and third quartiles are the margins of the box, while the line in the middle of the box is the median; the ends of whiskers are the difference between the first quartile and the 1.5 interquartile range, the sum of the third quartile and the 1.5 interquartile range

management of this cohort of pregnant women. Four models including polyamines assessed during the study showed high sensitivity and specificity (Fig. 4).

In the logistic regression model, plasma polyamine levels were independent variables, while the fact that the sample belonged to the PE or FGR group was a dependent variable. The area under the ROC curve for the model was 0.865; sensitivity and specificity were 0.95 and 0.76, respectively. The threshold values for the models constructed were 0.45, 0.3, 0.24, and 0.26.

DISCUSSION

Amino acids are the main building blocks for proteins playing an important role in fetal growth and development. Arginine, lysine, and aspartic acid not only take part in protein synthesis, but are also important for many biochemical pathways and body functions. Proline has an important function as a component of collagen, the main connective tissue protein. Its effects on the development of placenta and the fetus associated with the enhanced placental transport, angiogenesis, and protein synthesis are well known [18]. The decrease in blood levels of proline observed in pregnant women with FGR and early-onset PE that is directly correlated to the newborn's weight suggests that it can play a role in the pathogenesis of these pregnancy complications. Collagen is an important tissue component of the placental complex, and disruption of its structure in the phase of biosynthesis, secretion or assembly, caused by the proline level decrease, is likely to be one of the existing pathological mechanisms underlying realization of placental insufficiency. There is a report that cervical hydroxyproline concentration decreases in non-pregnant women having a history of cervical incompetence [19].

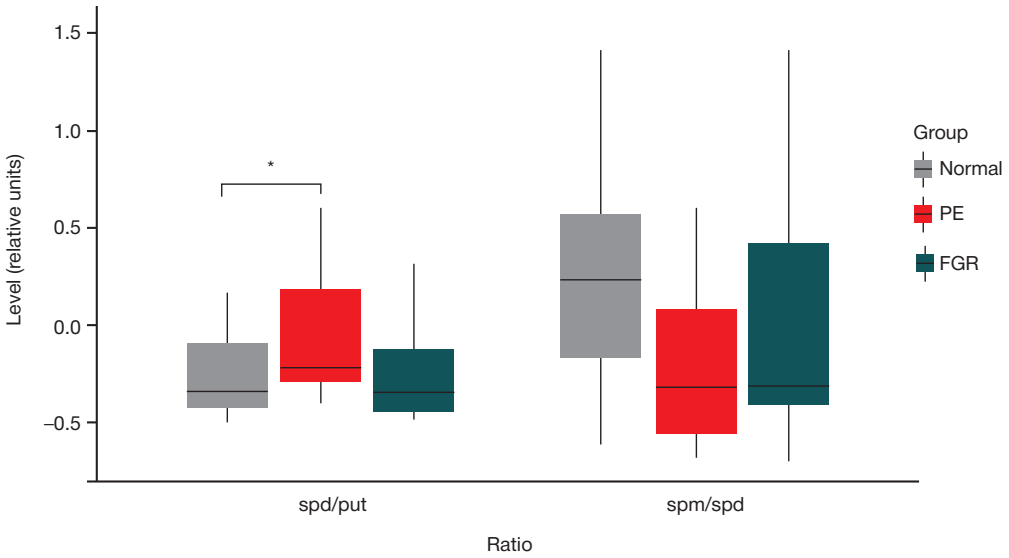

Fig. 3. Comparison of physiologically significant ratios of plasma polyamine levels. The first and third quartiles are the margins of the box, while the line in the middle of the box is the median; the ends of whiskers are the difference between the first quartile and the 1.5 interquartile range, the sum of the third quartile and the 1.5 interquartile range. spd/put — spermidine to putrescine ratio (spermidine synthase activity); spm/spd — spermine to spermidine ratio (spermine synthase activity)

Table 2. Significant correlation between clinical parameters and the levels of amino acids and polyamines (correlation coefficient, its confidence interval and significance (p))

Parameter		R	CI R	*p*
Amino acids				
Fetal condition decompensation in FGR	Proline	–0.56	–0.86 to –0.034	0.03
	Trans-4-hydroxyproline	–0.39	–0.59 to –0.066	0.01
Birth weight	Proline	0.67	0.041 to 0.87	0.02
	Trans-4-hydroxyproline	0.36	0.14 to 0.54	0.002
PE, severity	Glutamine	0.38	0.058 to 0.48	0.01
Length of hospital stay	Aspartic acid	–0.36	–0.5 to –0.088	0.007
Polyamines				
Fetal condition decompensation in FGR	1,7-diaminoheptane	–0.78	–0.92 to –0.37	0.002
	Cadaverine	–0.25	–0.46 to 0.013	0.04
PE, severity	Putrescine	–0.32	–0.52 to 0.083	0.009
Length of newborn's hospital stay	1,7-diaminoheptane	–0.76	–0.95 to 0.23	0.003

Along with the amino acids comprised in proteins, the body has a constant reserve of free amino acids contained in the tissues and various body fluids that are in dynamic equilibrium. During pregnancy free amino acids can serve as inducers of the synthesis of steroid hormones, take part in biosynthesis of glycoproteins, porphyrins, neurotransmitters, polyamines, and nitric oxide.

Metabolism of a number of amino acids results in the synthesis of regulatory polyamines. Polyamines are also found in various body fluids: blood, urine, cerebrospinal fluid, etc. The levels of free polyamines in blood are lower, than in the tissues and urine. However, it is determination of polyamines in blood of pregnant women as a marker of severity of such complications, as PE and FGR, that is of interest for practical medicine.

Assessment of the levels of free amino acids and polyamines can be important for understanding of molecular processes in pregnancy [20].

Our data demonstrate a significant correlation between alteration of blood polyamine levels and placenta-associated complications of pregnancy. Significant alterations were reported for such polyamines, as spermidine, putrescine, cadaverine, and 1,7-diaminoheptane, in the groups represented by pregnant women with placenta-associated complications. In FGR, the concentration of 1,7-diaminoheptane was significantly increased, against the background of the decrease in individuals with PE. Of particular interest is the fact that this feature observed in patients with FGR was previously reported in our paper on the diagnostic potential of urinary polyamines [21]. The results of our studies resonate with the study of the Chinese researchers, who have considered the effects of N1-guanyl-1,7-diaminoheptane (GC7), the potent deoxyhypusin synthase inhibitor, on proliferation, differentiation, and apoptosis of certain cells [22]. Involvement of diaminoheptanes in expression of cell proliferation proteins and apoptosis of endothelial cells suggests the possibility of using this biomarker for diagnosis of placenta-associated pregnancy complications and prediction of their severity. The increase in blood 1,7-diaminoheptane levels associated with FGR can be

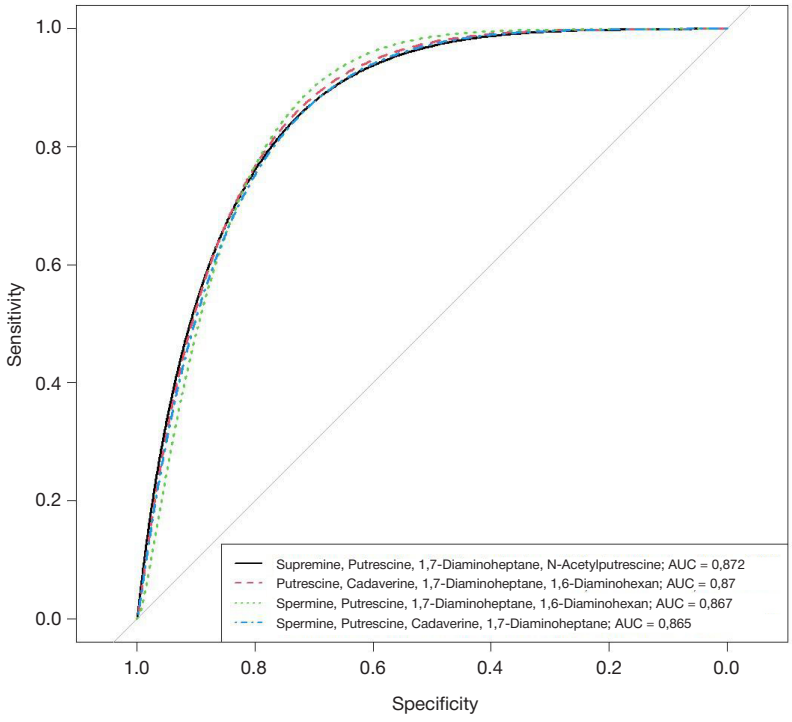

Fig. 4. ROC curves of the logistic regression models, in which plasma polyamine levels were independent variables, and the fact that the sample belonged to the PE or FGR group was a dependent variable. The insert shows amino acids, the levels of which a used as independent variables, along with the area under the ROC curve for appropriate logistic regression model

an indirect indicator of the dose-dependent inhibition of cell proliferation and a FGR biomarker, and change depending on the fetal condition severity increase.

Given the diversity and complexity of metabolic pathways responsible for adaptation under conditions of hypoxic damage, it should be expected that their regulation is coordinated. Metabolic pathways of low molecular weight oxidants, proline and polyamines, can be of particular interest in terms of studying regulation of such type.

Normally, homeostasis of proline and polyamines is maintained by the balanced system of their biosynthesis and degradation. The most common precursor in their biosynthesis is glutamate that is a more distant precursor first converted to ornithine or arginine. The resulting amino acids are the direct substrates of two enzymes (ornithine decarboxylase and arginine decarboxylase) catalyzing biosynthesis of putrescine, the precursor of longer polyamines (spermidine and spermine). Despite the determined link between the proline and polyamine synthesis pathways, the question about the possibility of coordinated regulation of metabolism of these low molecular weight compounds, especially in stressful conditions, remains debatable.

Analysis of the blood profile of free amino acids in placenta-associated pregnancy complications was of interest. According to some data, the levels of L-arginine, L-proline, and L-ornithine can reflect perinatal damage to the central nervous system in newborns with intrauterine growth restriction [23]. Significant correlations reported for a number of clinical parameters (fetal condition decompensation in FGR, PE severity, birth weight, length of stay in the hospital and in the neonatal pathology unit) and such amino acids, as proline and trans-4-hydroxyproline, glutamine and aspartic acid, suggest that these metabolites are involved in protein synthesis, maintenance of ribosome structure and collagen production in FGR, both isolated and combined with early-onset PE.

Considering the fact, that the data were collected in the phase of early clinical manifestations of obstetric syndrome, the possible potential of polyamines for prediction of the course of PE and FGR and the gestational age at delivery is incredibly high. The development of methods for diagnosis and monitoring of polyamine levels in pregnant women is of great interest. Such methods can provide instruments for prediction of the risk of obstetric syndromes and open the window of opportunity for further use of the methods after the in-depth validation and dynamic control depending on the course of FGR and early-onset PE over time.

CONCLUSIONS

The study of the polyamines' role and correlation with blood levels of free amino acids contributes to the development of methods for diagnosis and prediction of obstetric syndromes, which constitutes an important research area that can result in the emergence of new approaches to assessment and treatment of pregnant women, as well as in improvement of care of the maternal and child health in pregnancy.

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