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SOLUBLE ENDOGLIN AS AN EARLY PREDICTION MARKER OF INTRAUTERINE GROWTH RETARDATION

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High levels of circulating endoglin contribute to the development of endothelial dysfunction, which underlies the pathogenesis of intrauterine growth retardation and can be used as a prognostic marker for the development of this pathology. We collected serum from pregnant women with intrauterine growth retardation (n-41) initially at diagnosis and before delivery, and pregnant women with physiological pregnancy (n-8), using enzyme-linked immunosorbent assay to test soluble endoglin levels. It was found that the level of soluble endoglin concentration was 7.2±0.2 at the initial examination and 9.5±0.1 at the repeated examination in women who gave birth to low birth weight children with fetal developmental delay and constitutionally small children, while the level of this factor in women in the comparison group was 3.7±0.3. It was found that the level of soluble endoglin concentration was significantly higher in women who gave birth to children with intrauterine growth retardation than the level of this factor in women in the comparison group, which is highly informative to use its prognostic value.

Key words: soluble endoglin, fetal growth retardation, gestational age, low birth weight fetus.

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РОЗЧИННИЙ ЕНДОГЛІН ЯК МАРКЕР РАННЬОГО ПРОГНОЗУВАННЯ ЗАТРИМКИ ВНУТРІШНЬОУТРОБНОГО РОЗВИТКУ ПЛОДА

Високий рівень циркулюючого ендогліну сприяє розвитку ендотеліальної дисфункції, яка лежить в основі патогенезу затримки внутрішньоутробного розвитку плода і може бути використаний як прогностичний маркер розвитку даної патології. Проведений забір сироватки крові у вагітних із затримкою внутрішньоутробного розвитку плода (n-41) первинно при встановленні діагнозу та перед розродженням, та вагітних з фізіологічним перебігом вагітності (n-8), яким імуноферментним методом дослідили рівень розчинного ендогліну. Виявлено, що рівень концентрації розчинного ендогліну був 7.2±0.2 при обстеженні первинно та 9.5±0.1 при повторному обстеженні у жінок, які народили маловагових дітей із затримкою внутрішньоутробного розвитку плода та конституційно малих дітей, натомість рівень даного фактору у жінок групи порівняння був 3.7±0.3. Виявлено, що рівень концентрації розчинного ендогліну був достовірно вищий у жінок, які народили дітей із затримкою внутрішньоутробного розвитку плода, ніж рівень даного фактору у жінок групи порівняння, що з високою інформативністю дозволяє використовувати його прогностичну значущість.

Ключові слова: розчинний ендоглін, затримка внутрішньоутробного розвитку плода, термін вагітності, мало ваговий плід.

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Intrauterine growth retardation (IUGR) is an obstetric pathology that remains the second key cause of preventable perinatal morbidity and mortality. This pathology is relevant for many fields of medicine, namely obstetrics, gynecology, neonatology, and pediatrics. A more in-depth study of this complication of pregnancy is relevant for effective detection in early pregnancy. At early detection of IUGR effective management of pregnancy and a choice of the optimum term of delivery is possible [11].

The physiological development of pregnancy depends on the normal development of the fetal-placental complex. Angiogenic growth factors stimulate proliferation, trophoblast invasion and are responsible for the growth, development of spiral arteries of the uterine-placental complex. The placenta also in turn produces and secretes into the maternal circulation angiogenic growth factors, namely placental growth factor, transforming growth factor β, and their soluble co-receptors FMS-like thyroxine kinase-1 and soluble endoglin [12].

Endoglin (Eng) is an important angiogenic factor that is an isoform of the TGF-β signaling complex co-receptor, which is mainly expressed on endothelial cells and plays a key role in angiogenesis. It is well known that its extracellular molecule can be cleaved and released into the bloodstream in the form of soluble Eng (s-Eng) [5]. This angiogenic protein, spreading throughout the mother's body, causes a massive spread of epithelial damage, culminating in multisystem organ damage. High levels of circulating s-Eng contribute to the development of endothelial dysfunction, which underlies the pathogenesis of such complications of pregnancy as IUGR, preeclampsia, premature birth, antenatal fetal death [2,9].

During pregnancy, angiogenic factors play a key role in the overall remodeling of the vascular network of the uterus. The placenta alone produces several factors with proangiogenic and antiangiogenic activity. For the most part, the regulation of the expression and secretion of these factors is necessary for physiological placentation, the mother's immune adaptation to pregnancy, and subsequently for the growth

and development of the fetus. From this, we can conclude that a high level of angiogenic factor, s-Eng, in pregnant women in the first trimester is associated with subsequent IUGR and preeclampsia. With a decreased rate of decrease in s-Eng, between the first and second trimesters of pregnancy, women had a higher risk of adverse pregnancy termination, including IUGR. Thus, at elevated plasma concentrations during the second and third trimesters of pregnancy, mothers gave birth to underweight children [4].

Considering that preeclampsia and IUGR have the same angiogenic profiles and the same pathogeneses, it can be concluded that the profiles of angiogenic markers are not determined by specificity for the individual identification of IUGR and preeclampsia. Elevated levels of s-Eng can be included in the profile of biomarkers to predict not only preeclampsia but also IUGR [1].

The study of angiogenic factors is important in the pathogenesis of IUGR. It is known that the pleiotropic cytokine TGF-β1 is mainly produced by leukocytes, showing mainly anti-inflammatory effects, and thus helps maintain immune tolerance and endothelial integrity. s-Eng is a soluble TGF-β receptor that binds TGF-β and reduces its bioavailability. s-Eng inhibits the immunoregulatory effect of TGF-β and acts as an antiangiogenic factor in the placenta, inhibiting vascular permeability and nitric oxide-mediated vasodilation [6].

The purpose of the study was to determine the level of soluble endoglin in the serum of women whose pregnancy was complicated by intrauterine growth retardation.

Materials and methods. In accordance with the set goal, blood was taken from pregnant women with intrauterine growth retardation, who were included in the main group (n=41) and pregnant women with physiological pregnancy – control group (n=8), in which enzyme-linked immunosorbent assay was used to examine the level of s-Eng. Women of the main group underwent blood sampling immediately after ultrasound identification of IUGR. The level of s-Eng was assessed in women of the main group based on the dynamics of pregnancy progression: initially – immediately after the establishment of IUGR on ultrasound and again – prior to delivery. In women of the control group, it was determined once, after ultrasonography at 30–35 weeks of pregnancy. Depending on the weight of the children born, the main group was divided into the first subgroup – 25 women who gave birth to low birth weight infants and the second subgroup – 16 women who gave birth to constitutionally small children.

The criterion for inclusion in the study was women with spontaneous singleton pregnancies, who were diagnosed with IUGR by ultrasound. The criterion for exclusion from the study was severe extragenital pathology in pregnant women.

Elabscience Biotechnology Inc. serum was examined to determine soluble endoglin. ELAB-EK-L2AFH8APSS. S-Eng ELISA set № E-EL-H5309; 96T (USA). Human endoglin is an enzyme-linked immunosorbent assay for the quantitative determination of human endoglin in the cell culture supernatant, serum, blood plasma.

Statistical analysis of the results of the study was calculated using the program "MedStat" methods of descriptive statistics, calculating the average sample values (M) and the error of the mean value (m). The Student's t-test or Mann-Whitney U test was used to assess intergroup differences. Spearman's R or Kendall's τ, logistic regression method, was used to determine rank correlation, and ROC analysis was used to assess diagnostic efficiency. ROC analysis data are presented as the area under the curve (AUC) with a 95 % confidence interval (CI). For analysis, the differences were considered statistically significant at $p < 0.05$.

Results of the study and their discussion. In order to establish the prognosis of the angiogenic factor, regulating the mechanism of development and functioning of the placenta during pregnancy, the level of s-Eng was determined.

We found (table 1) that in women of the main group, with the progression of pregnancy the level of s-Eng concentration significantly increased 1.3 times (p=0.00001) compared with the initial level of indicators.

Table 1

Indices of soluble endoglin (s-Eng) levels in women of the main group and the control group

Index	Main group n=41		Comparison group n=8	p1	p2
	initial	repeated			
s-Eng, ng/ml	7.2±0.2	9.5±0.1	3.7±0.3	0.000001	0.000001

Notes: p1 – when comparing the initial and redetermined indicators; p2 – when comparing indicators in the main group and the control group.

It was also found that the level of s-Eng concentration was significantly 2.6 times higher in women of the main group than in women of the control group (p=0.00001).

In order to determine the change in the level of angiogenic factor concentration at the initial diagnosis of fetal growth retardation and before delivery depending on the gestational age, we analyzed the s-Eng in both groups of pregnant women.

In women of the main group, it was found that initially after ultrasound identification of IUGR, the levels of s-Eng indicators gradually increased in accordance with the increase in gestational age, at which the level of the indicator was determined. The lowest level of s-Eng was established at 27 weeks of pregnancy (5.1 ng/ml) and the highest – at 30 weeks of pregnancy (7.9 ng/ml).

The level of s-Eng concentration in the main group (Fig.1) in women with IUGR, who gave birth before 36+6 weeks was initially determined to be 7.5±0.2 ng/ml, and at redetermination 9.2±0.2 ng/ml. In women with IUGR, who gave birth after 37 weeks the initial level was 6.9±0.2 ng/ml, and at redetermination 9.8±0.2 ng/ml.

It was found that the level of s-Eng concentration at the initial determination was significantly higher, and at redetermination, on the contrary, significantly lower in women with IUGR who gave birth before 36+6 weeks than in women with IUGR who gave birth after 37 weeks (p=0.04; p=0.004, respectively).

So, the determination of a significantly higher level of s-Eng concentration in the initially established ultrasound diagnosis of IUGR may have prognostic value for early assessment of the risks of premature birth, namely up to 36+6 weeks of pregnancy.

Today, the existing predictors of the development of IUGR have low prognostic significance, as they reflect only certain mechanisms of pathological changes in the formation of small or sufficient weight by gestational age of the child on the background of the development of IUGR. To increase the informativeness of the prognosis of early detection of a child with developmental delay, it is necessary to look for a multi-component approach to prognosis. Using the methods of logistic regression with ROC-analysis, it is possible to calculate the prognostic significance of the studied angiogenic marker – s-Eng.

The level of concentration of s-Eng (fig. 2) in women with IUGR who gave birth to low birth weight infants was at initial determination 7.3±0.2 ng/ml, and at repeated – 9.3±0.1 ng/ml, and in women whose pregnancy was complicated by IUGR and gave birth to constitutionally small children was at the initial determination 6.9±0.3 ng/ml, and at repeated – 9.8±0.2 ng/ml.

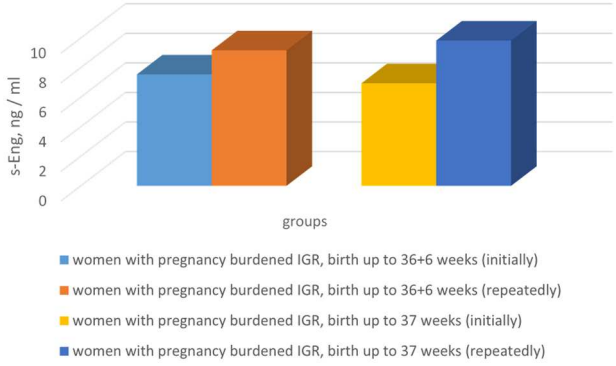

Fig. 1. Indices of the s-Eng level in the serum of women with pregnancy burdened IUGR, birth up to 36+6 weeks (initial and repeated) and women with pregnancy burdened IUGR, birth after 37 weeks (initial and repeated).

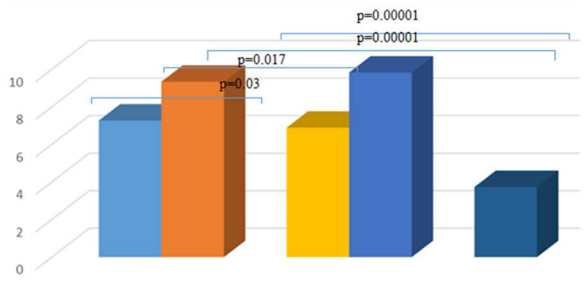

Fig.2. Indices of the s-Eng level in the serum of women with pregnancy complicated by IUGR, who gave birth to low birth weight children (initial and repeated) and constitutionally small children (initial and repeated), women of the control group.

In the dynamics, a significant increase in the level of s-Eng was observed in women whose pregnancy was complicated by IUGR and gave birth to low birth weight children, because its concentration level was significantly 1.3 times lower at the initial than at the repeated determination (p=0.00001).

Similarly, the dynamics showed a significant increase in the level of s-Eng in women whose pregnancy was complicated by IUGR and gave birth to constitutionally small children, because its concentration level was significantly 1.4 times lower in the initial determination than in the second (p=0.00001).

There was a significantly higher level of s-Eng in women who gave birth to low birth weight children with IUGR than in women who gave birth to constitutionally small children at the initial determination (p=0.017, Wilcoxon test).

However, a significantly lower level of s-Eng was found in women who gave birth to low birth weight children with IUGR than in women who gave birth to constitutionally small children at redetermination (p=0.03, odd t-test).It was found that the level of pH concentration was significantly higher

in women who gave birth to low birth weight children with IUGR and constitutionally small children than the level of this factor in women of the control group (p=0.00001, p=0.00001, respectively).

So, the obtained data summarize the difference between the two groups of women whose pregnancy was complicated by IUGR with the birth of underweight and constitutionally small children with a key emphasis on impaired synthesis and functioning of s-Eng. There was a significantly higher level of s-Eng in the identification of IUGR in pregnant women who gave birth to low birth weight children than in pregnant women who gave birth to constitutionally small children. At the same time, the level of s-Eng before birth is significantly lower in pregnant women who gave birth to low birth weight children with IUGR than in women who gave birth to constitutionally small children.

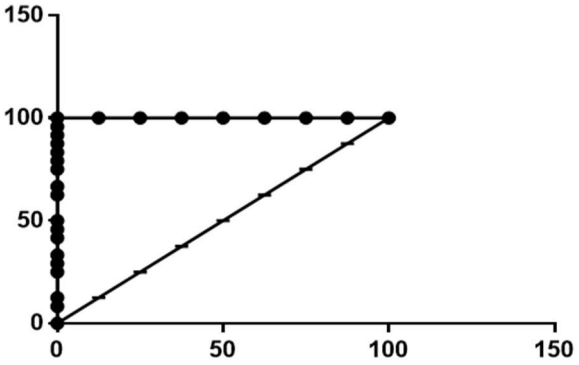

Fig. 3. Analysis of ROC-curve levels of s-Eng in the serum of pregnant women to differentiate the group of children born with low birth weight.

To determine the significance of s-Eng as a diagnostic prognostic test, we conducted a ROC-analysis (Fig. 3), which described the obtained model as satisfactory and justified the prospects of using it at the initial stages of identification of IUGR to determine the birth of low birth weight children:

• S-Eng (AUC=1.0; Std. Error=0; 95 % CI 1–1; p<0.0001). The quality of prediction of early detection of low weight at the distribution point d=4.5 with a sensitivity of 100 % and a specificity of 75 %, the value of d>4.5 – indicates a high risk of IUGR;

So, based on the data we obtained, we can conclude that determining the level of s-Eng in the serum of pregnant women with high informativity allows using its prognostic value in the risk of low birth weight in pregnant women with IUGR.

A regression analysis was performed to determine the indicators that are crucial in predicting low birth weight in women whose pregnancies are complicated by IUGR. In the step-by-step discriminant analysis, we included the indicators of the s-Eng and the indicators of the ultrasound examination.

In the examined group, a functional relationship was found between the parameters of the weight of a newborn with low weight for gestational age and changes in pregnancy and s-Eng levels, which are described by the linear regression equation:

Newborn weight=106,582 × gestational age – 172.87×s-Eng, provided that d s-Eng <6.9 – this model increases the sensitivity of the method.

According to the ROC-analysis according to the s-Eng indicator, a decrease in the quality of predicting early detection of low weight at the distribution point d=6.85 with a sensitivity of 66.7 % and a specificity of 100 %, the value of d <6.9 – indicates an increase in the quality of the method in predicting whose pregnancy is complicated by IUGR, which must be taken into account in our proposed model for assessing the weight of the newborn. Therefore, our proposed formula allows you to most effectively predict the weight of the newborn.

Our studies are consistent with the work of several authors, which show that in pregnant women with PE and IUGR higher s-Eng level directly correlates with premature birth before 34 weeks of gestation, and the severity of the pathology is an independent marker of risk of low birth weight for gestational age [7, 8]. It is possible to suggest that intrauterine inflammation associated with immaturity and low birth weight may cause an antiregulatory anti-inflammatory response, leading to increased s-Eng synthesis and the development of endothelial dysfunction with partial compensation [10]. It was also found that the level of s-Eng concentration in the serum has a prognostic value at the time of diagnosis of IUGR, at 24–34 weeks of pregnancy, especially in women whose childbirth occurs before 34 weeks of pregnancy [3].

During pregnancy, angiogenic factors play a major role. From this, we can conclude that a high level of angiogenic factor, s-Eng, in the mother in the first trimester of pregnancy is associated with subsequent preeclampsia and IUGR. Decrease in rate of decrease of s-Eng, between the first and second general remodeling of a vascular network of a uterus. Only the placenta produces several factors with proangiogenic and antiangiogenic activity. Regulation of the expression and secretion of these factors is necessary for physiological placentation, an immune adaptation of the mother to pregnancy, and subsequently for the development and growth of the fetus during the trimesters of pregnancy, mothers with a higher risk of adverse pregnancy, including IUGR. In contrast, at elevated plasma concentrations during the second and third trimesters of pregnancy, mothers gave birth to low birth weight infants for gestational age [5,7].

Raia-Barjat T. and co-authors, studying the early and late forms of IUGR, determined the level of s-Eng at different terms of pregnancy. Plasma levels were increased in women with IUGR compared to women with physiological pregnancy at 28, 32, and 36 weeks of pregnancy. Pregnancy, complicated by early and late onset of IUGR, and late preeclampsia, had high angiogenic factors at 32 and 36 weeks of gestation. On the other hand, when comparing IUGR with preeclampsia, the level of s-Eng in women's plasma did not change significantly. This study proves that s-Eng can be a predictor for early diagnosis of IUGR [9]. The same view is shared by other researchers, who believe that considering the similar angiogenic profiles and the pathogeneses of preeclampsia and IUGR, it can be concluded that the profiles of angiogenic markers are not determined by the specificity to individually identify IUGR, and preeclampsia. Elevated levels of s-Eng can be included in the profile of biomarkers for predicting not only preeclampsia but also IUGR [1,10].

Conclusions

1. Impaired expression of angiogenic factors plays an important role in the pathogenetic mechanism of IUGR.

2. Soluble endoglin affects the processes of angiogenesis during placentation in physiological pregnancy.

3. The formation of IUGR is accompanied by an increased level of s-Eng in the blood of pregnant women, which indicates a violation of angiogenesis and the development of placental dysfunction.

4. Determination of the level of s-Eng in the blood of pregnant women can be recommended as an early diagnostic marker not only of IUGR, but also of the premature birth of children.

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