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Research Article

Clinical and Pathogenetic Causes of Developing Complications in Multiple Pregnancy

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Abstract
To assess the role of the placental growth factor in the development of gestational complications during multiple pregnancy, there was conducted a study of this indicator in serum of 320 pregnant women with multiple pregnancy in the first trimester and 40 pregnant women with singleton pregnancy (the control group).
The objective of the research was to study the effect of placental growth factors on the gestational process in multiple pregnancy.
Materials and Methods. There was conducted a prospective study of pregnancy and childbirth in 320 females with multiple pregnancy (the main group) and 40 healthy women with singleton pregnancy. The level of serum placental growth factor was determined by enzyme-linked immunosorbent assay using monoclonal antibodies in the first trimester of pregnancy. The indicators of the hemostasis system (vascular, platelet and coagulation components) were evaluated according to generally accepted methods. Doppler ultrasound of the placental and fetal blood flow was performed in the uterine arteries, the umbilical artery and vein, the fetal middle cerebral artery.
Results. Women with multiple pregnancy were at high risk of developing gestational complications such as preterm deliveries (67.8%, $p < 0.01$), placental dysfunction, pre-eclampsia (17.5%, $p < 0.05$). The disorders of the vascular platelet and coagulation hemostasis in the first trimester of pregnancy were the main risk factors for early termination of pregnancy. Low level of serum placental growth factor in pregnant women with multiple pregnancy in case of preterm delivery, placental dysfunction and pre-eclampsia (111.23 ± 8.4, 203.24 ± 6.4 and 305.86 ± 7.4 pg/ml), in comparison with the corresponding indicators in singleton pregnancy (418.2 ± 10.4 pg/ml), was proven to be a prognostic marker for the development of gestational complications.
Conclusions. Timely correction of gestational complications in multiple pregnancy with micronized progesterone, low molecular weight heparins, angio-protective agents allowed us to prolong pregnancy with monochorionic placentation type for 3.2 weeks (up to 34.2 ± 2.4 weeks) and provide full-time delivery of dichorionic twin pregnancy.

Keywords
multiple pregnancy; miscarriage; preterm delivery; placental dysfunction; placental growth factor; pre-eclampsia

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Problem statement and analysis of the latest research

The vast majority of clinical studies proved multiple pregnancy to be accompanied by a significant number of perinatal complications, namely premature termination of pregnancy, placental dysfunction with fetal growth restriction, and pre-eclampsia, which, in turn, contributes to increased perinatal morbidity and mortality [1, 2, 7]. Perinatal mortal-

ity remains higher in multiple pregnancy as compared to singleton pregnancy, and the risk of antenatal fetal death in twin or triplet pregnancy is ten times higher [2, 6, 7]. The course of multiple pregnancy is predicted and further depends on the process of implantation of the fetal egg, the type of placenta, the adaptation of the mother's body to pregnancy, and environmental influences on the pregnant woman during the critical periods of fetal development.

According to statistics, the incidence of pre-eclampsia in multiple pregnancy is 3 to 4 times higher than in singleton pregnancy. The risk of pre-eclampsia in multiple pregnancy increases due to hyperplacentosis; the impaired perfusion of the placenta results in placental insufficiency and inadequate blood flow [4, 8]. One of the most common hypotheses concerning the pathogenetic mechanisms of pre-eclampsia development includes primary disturbance of the uteroplacental blood flow and concomitant impairment of the invasion of the myometrial spiral arteries by cytotrophoblast, superficial trophoblastic invasion followed by incomplete remodeling of the vascular structures where a slowdown of the blood flow due to increased vascular resistance is observed. Incomplete invasion results in impaired placental angiogenesis and, thus, plays a key role in the development of pre-eclampsia. The impaired development of the chorionic villi is known to be observed when maternal plasma concentration of the placental growth factor (PlGF), that belongs to the family of endothelial growth factors, decreases [3, 4, 9, 10]. The gene, that encodes the synthesis of this factor, is proven to be localized on the long arm of chromosome 14. PlGF is produced by trophoblast, namely endothelial cells of trophoblastic and placental macrophages [5]. PlGF possesses pronounced angiogenic potential, thereby promoting angiogenesis. PlGF is a glycosylated homodimer, the biological effect of which is realized by activating the vascular receptors with subsequent stimulation of vasculogenesis and angiogenesis. The decrease in the concentration of PlGF in the trophoblastic tissues undoubtedly leads to the impaired development of the chorionic villi, reduction in nutrient and oxygen delivery to the fetus, thereby creating a model of placental dysfunction with subsequent fetal growth restriction [3]. PlGF enters the mother's bloodstream along with numerous hormones and peptides that are secreted by the cyto- and syncytiotrophoblasts [4]. In this regard, PlGF should be considered as a marker for a number of gestational diseases, namely pre-eclampsia, placental insufficiency and fetal growth restriction. Slow blood flow provokes endothelial damage, promotes vascular microthrombosis and placental infarctions. Due to increased vascular permeability, a cascade of hemodynamic disturbances is triggered, which results in the disruption of regulatory mechanisms and the development of chronic disseminated intravascular coagulation and decompensated placental insufficiency. The generalization of endothelial dysfunction process is associated with the release of cytokines, the formation of free radicals, acidosis [4, 10].

The study of the immunological processes ensuring normal pregnancy and the identification of the pathogenetic mechanisms leading to its impaired physiological course are one of the primary tasks of reproductive immunology [5]. Normal functioning of the fetal-placental complex is essential for gestation. At the stage of placental formation, PlGFs play the main role in the regulation processes, ensuring normal functioning of the mother-placenta-fetus system. PlGFs contribute to cytotrophoblast invasion; impaired angiogenesis and invasion processes of the spiral arteries are directly related to their insufficiency and provoke the development of gestational complications, namely placental dysfunction, miscarriage and pre-eclampsia progression [1, 6].

The objective of the research was to study the effect of PlGFs on the gestational process in multiple pregnancy.

1. Materials and Methods

The study was conducted in the Sumy Regional Clinical Perinatal Center and the Department of Obstetrics and Gynecology of the Sumy State University during 2012-2017. There was carried out a prospective study of pregnancy and childbirth in 320 females with multiple pregnancy. Among them,

there were 144 females with monochorionic diamniotic twin pregnancy (Group I) and 176 women with dichorionic diamniotic twin pregnancy (Group II). The control group consisted of 40 healthy women with singleton pregnancy. The examined groups were homogeneous in composition and representativeness. The average age of pregnant women in the main group was 29.8 $\pm$ 4.5 years, while in the control group, it was 27.6 $\pm$ 3.2 years. There were no differences in age, somatic pathology, frequency of obstetric complications between the control group and the main groups. Laboratory studies were carried out in the clinical laboratory of the Sumy Clinical Regional Perinatal Center, the Synevo Laboratory, and the Medical and Genetic Laboratory of the Sumy State University.

Serum PlGF level was determined by enzyme-linked immunosorbent assay using monoclonal antibodies and standard R& D system reagents in the first trimester of pregnancy. The state of the fetal-placental complex was evaluated during real-time ultrasound scanning by means of modern PHILIPS Sono Diagnost 360 Ultrasound Machine (Netherlands) and Aloka SSD-2010 Ultrasound Machine (Japan) with a 6.5-MHz transvaginal probe in the early stages of pregnancy and 3.5- and 5-NHz convex probes in the late stages of pregnancy using two-dimensional imaging. It included the determination of chorionicity, the state and amount of amniotic fluid, placentometry, fetometry, Doppler ultrasound. Doppler ultrasound of the placental and fetal blood flow was performed in the uterine arteries, the umbilical artery and vein, the fetal middle cerebral artery. The following parameters were measured: the peak blood flow velocity during systole and early ventricular diastole, the mean blood flow velocity over the cardiac cycle, the pulsation index, the resistive index and the systolic/diastolic ratio. The indicators of the hemostasis system (vascular, platelet and coagulation components) were evaluated according to generally accepted methods.

Statistical processing and analysis of the data obtained were carried out using Microsoft Excel programs by means of mathematical statistics methods and the Statistika 8.0 software package. Charts and diagrams were created using Microsoft Excel.

2. Results and Discussion

According to the research results, a history of chronic inflammatory diseases, as well as the exacerbation of infections in the first trimester of pregnancy, when implantation processes, trophoblastic invasion, further development and normal functioning of the fetal-placental complex take place, had a significant impact on the course of multiple pregnancy and the development of gestational complications. According to our data, only in 43 (13.4%) women with multiple pregnancy, a physiological course of gestation was seen. Termination of pregnancy up to the 12^{th} week of gestation was observed in 17 (5.3%) patients with multiple pregnancy, and reduction of one fetus was observed in 8 (2.5%) pregnant women. The course of multiple pregnancy was accompanied by the symptoms of threatened abortion in 180 (56.2%) cases, which resulted in inpatient treatment in the periods between 8 and 12, 14 and 16, 18 and 22, 24 and 28 weeks of gestation. Among the main etiologic factors that provoked threatened abortion, a history of chronic endometritis ranked first in 107 (33.4%) pregnant women, while in the control group, this cause was diagnosed in 24 (7.5%) pregnant women only. Microscopic examination of endocervical and vaginal discharge revealed opportunistic microorganisms in 168 (52.6%) patients of the main group, and 5 (12.5%) patients of the control group. Viral and bacterial co-infection (Epstein-Barr virus with coexistent Ureaplasma urealiticum) was detected in the cervical cultures of 107 (33.4%) patients of the main group and 3 (7.5%) patients of the control group; pathogenic staphylococcus and streptococcus were detected in 60 (18.7%) pregnant women of the main group and 2 (5.0%) pregnant women of the control group.

The study of the hemostasis system in the women with multiple pregnancy proved changes to occur in the first trimester of pregnancy already. These abnormalities were manifested by characteristic changes in the vascular platelet component: a platelet count was 181.2 $\pm$ 8.7 $\times$ 10^9/l with the control group indicator of 236.4 $\pm$ 8.7 $\times$ 10^9/l; there was observed an inhibition of adenosine diphosphate-

induced platelet aggregation and its reduction by 1.2 times (32.4 $\pm$ 1.7%) in almost half of pregnant women in the main group as compared to the average indicator of the control group - 56.2 $\pm$ 2.6% ($p < 0.01$). The study of the coagulation component showed an increase in fibrinogen level to 5.5 $\pm$ 0.6 g/l in the main group with the control group indicator of 2.89 $\pm$ 0.8 g/l ($p < 0.05$); the increase in plasma level of soluble fibrin-monomer complex up to 12.0 $\pm$ 1.2 mg/100 ml in the main group with the control group indicator of 4.8 $\pm$ 0.6 mg/100 ml ($p < 0.05$); shortening of the activated partial thromboplastin time to 26.4 $\pm$ 1.2 sec as compared to the control group - 31.8 $\pm$ 2.2 sec, ($p < 0.05$). The results of studying the vascular platelet and coagulation components of hemostasis indicated an increase in blood coagulation in pregnant women of the main group, which, in turn, served as one of the pathogenetic mechanisms of developing placental dysfunction and pre-eclampsia. In this regard, there was a slowdown of the blood flow in the intervertebral space followed by the increase in local pressure, which impeded the blood flow in the spiral arteries and led to the development of placental ischemia. When performing Doppler ultrasound in 23 (7.2%) patients of the main group, there was detected a reverse blood flow in the umbilical artery which was not observed in the control group. A single fetal death was seen in 8 pregnant women of the main group. As a result of our study, we found hemodynamic disturbances in multiple pregnancy to be accompanied by growth retardation of one fetus in 105 (32.8%) pregnancies, or both fetuses in 56 (17.5%) pregnancies.

Disturbances of the fetoplacental blood flow along with changes in rheological and coagulation properties of blood in multiple pregnancy result in higher frequency of gestational complications, such as miscarriage, placental dysfunction and fetal growth restriction, pre-eclampsia, as compared to the indicators in singleton pregnancy. Due to disturbance of the uteroplacental blood flow caused by morphofunctional changes in the spiral arteries and intervertebral space, in the main group, preterm births occurred in 217 (67.8%) cases ($p < 0.01$), severe forms of pre-eclampsia developed in 56 (17.5%) cases ($p < 0.01$); in the control group, preterm births and pre-eclampsia were seen in 3 (7.5%) and 1 (2.5%) cases, respectively

The study of prognostic factors for gestational complications in multiple pregnancy, that lead to its premature termination, confirmed the role of PlGF in the regulation of trophoblastic invasion processes. The analysis of serum PlGF level in pregnant women in the main group revealed its decrease to 111.23 $\pm$ 8.4 pg/ml in case of preterm delivery and to 203.24 $\pm$ 6.4 pg/ml in case of placental insufficiency; in pregnant women with pre-eclampsia, this indicator was 305.86 $\pm$ 7.4 pg/ml as compared to the indicator of the control group - 418.2 $\pm$ 10.4 pg/ml ($p < 0.01$).

The study of dynamic changes in serum PlGF levels in pregnant women allowed us to develop an algorithm for early prevention of gestational complications in multiple pregnancy in the periods between 8 and 14, 16 and 18, 22 and 24, 30 and 32 weeks of gestation. The scheme of preventive treatment necessarily included micronized progesterone (lutein), nitric oxide donors (tivortin), disaggregants (acetylsalicylic acid), and anticoagulants - low molecular weight heparins (zibor) according to risk factors and mandatory dynamic monitoring of blood parameters. Starting from the 14^{th} week of pregnancy, taking into account clinical and laboratory parameters, there was used the angio-protective agent diosmin (Phlebodia) that has a high degree of tropism for the vessels and allows improving the state of the vascular wall, helps eliminate angiopathy in the spiral arteries and normalize hemodynamic processes in the fetal-placental complex. Timely correction of existing clinical and laboratory disorders allowed us to prolong pregnancy with monochorionic placentation type for 3.2 weeks (up to 34.2 $\pm$ 2.4 weeks), and provide the delivery of full-term dichorionic twins.

Thus, summarizing the research results, it should be noted that the determination of PlGF level in pregnant women with multiple pregnancy can be used as an early criterion for predicting gestational complications. Early treatment and prophylactic measures in pregnant women with multiple pregnancy in critical terms allowed us to prolong preg-

nancy and improve perinatal effects as much as possible.

3. Conclusions

1. Women with multiple pregnancy were at high risk of developing gestational complications such as preterm deliveries (67.8%, $p < 0.01$), placental dysfunction, pre-eclampsia (17.5%, $p < 0.05$).
2. Low serum PlGF level in pregnant women with multiple pregnancy in case of preterm delivery, placental dysfunction and pre-eclampsia (111.23 ± 8.4, 203.24 ± 6.4 and 305.86 ± 7.4 pg/ml), in comparison with the corresponding indicators in singleton pregnancy (418.2 ± 10.4 pg/ml) is a prognostic marker for the development of gestational complications as reliably confirmed by the study ($p < 0.01$).
3. The disorders of the vascular platelet and coagulation hemostasis revealed in the first trimester of pregnancy are the main risk factors for early termination of pregnancy.
4. Timely correction of gestational complications in multiple pregnancy with micronized progesterone, low molecular weight heparins, angio-protective agents allowed us to prolong pregnancy with monochorionic placentation type for 3.2 weeks (up to 34.2 ± 2.4 weeks) and provide full-time delivery of dichorionic twin pregnancy.

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