JSC “WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY”

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY №2

ANNOTATION

The dissertation thesis «Preeclampsia associated with the level of placental growth factor and the polymorphism rs1042886 of the *PlGF* gene in Kazakh women»

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Introduction. Preeclampsia is a multisystem disorder that complicates 4-8% of all pregnancies and is a leading cause of maternal and neonatal morbidity and mortality [1,2,3,4]. Despite the fact that intensive research is underway in this area, the pathophysiology of preeclampsia is still unclear. One of the most common hypotheses for the onset of preeclampsia is superficial invasion of the extravillous trophoblast followed by incomplete remodeling of the mother's vascular structures, which leads to uteroplacental insufficiency and intrauterine growth retardation [5,6,7,8].

In today, the significance of pro- and anti-angiogenic factors as early serum predictors of preeclampsia is best studied [9].

Placental growth factor (PLGF) belongs to the family of vascular endothelial growth factor (VEGF) and is one of the most important regulators of placenta formation and vascularization of its villi [10]. In the human body, PLGF is represented by 4 isoforms: PLGF-1, PLGF-2, PLGF-3, PLGF-4 [11]. An imbalance of angiogenic factors, such as vascular endothelial growth factor (VEGF) or placental growth factor (PLGF), and factors inhibiting angiogenesis, such as soluble fms-like tyrosine kinase-1 (sFLT-1), plays a fundamental role in the pathogenesis of preeclampsia [12,13].

A decrease in the concentration of free PLGF and an increase of sFlt-1 in pregnant women is a marker of defective placentation and preeclampsia. Despite the fact that PLGF plays a significant role in the normal course of embryogenesis, there is little information on pregnancy pathologies associated with the presence of a single nucleotide polymorphism (SNP) of this gene in the genome. The PLGF gene is located on chromosome 14q24.3. SNP of the PLGF gene can be associated with the development of pathology of placentation and embryogenesis. For instance, of PLGF rs1042886 polymorphism is associated with preeclampsia among the female population of Sri Lanka [1,14]. Muetze S. et al. in their study looked for the connection of point mutations of the PLGF, Flt-1, IGF-I, and IGF-IR genes with intrauterine growth retardation, however, no such connection was found in any of the genes [14,15].

The presence of gene polymorphisms plays an important role in the development of preeclampsia. The study of the genetic predisposition to preeclampsia will allow at the pregravid stage to predict and timely form a high-risk group for the development of preeclampsia. But while this prognostic mechanism is under study [16].

Despite some advances in the study of the molecular mechanisms of preeclampsia, it should be noted that the results obtained by different researchers in the study of the genetic predisposition to this disease are often contradictory for certain ethnic groups. Some authors consider the variability of the structure of the hereditary component of preeclampsia between different population samples, which is formed as a result of the demographic history of a particular population, as the main reason for this kind of contradictions.

Thus, it seems extremely important to study the structure of hereditary predisposition to preeclampsia, taking into account ethnicity and to search for common and ethnospecific genetic markers of this pathology [17].

The possibility of identifying pregnant women in the risk group for the development of early preeclampsia, determination of the level of genetic markers will significantly increase the possibility of more accurate prediction of the development of early preeclampsia. And this, in turn, will make it possible to timely begin the prevention of complications from both the mother and the fetus. Therefore, it becomes necessary to study this particular topic.

Purpose of the study:

To assess the relationship between SNP rs1042886 of the PLGF gene and preeclampsia in Kazakh women and to study the clinical significance of PLGF as a potential prognostic marker of preeclampsia.

Research Objectives:

1. To determine the level of placental growth factor (PLGF) in the first trimester of pregnancy in Kazakh women.
2. To determine the relationship between the development of preeclampsia and the level of PLGF in the first trimester of pregnancy in Kazakh women.
3. To determine the relationship between SNP rs1042886 PLGF and the development of preeclampsia in Kazakh women.

The scientific novelty of the dissertation research:

1. The rs1042886 polymorphism of the PLGF gene in Kazakh women was studied for the first time.
2. For the first time, the relationship between the development of preeclampsia and the level of PLGF in blood and urine in the first trimester of pregnancy in Kazakh women was studied.
3. For the first time, the relationship between the rs1042886 PLGF polymorphism and the development of preeclampsia in Kazakh women was studied.

Theoretical and practical significance: a model for predicting preeclampsia in early pregnancy obtained as a result of mathematical modeling, including the results of clinical and laboratory monitoring of the levels of placental growth factor in blood and urine, which can be used to assess the prognosis of pregnancy outcome in Kazakh women was developed.

Approbation of work. The main provisions on dissertation were reported at an expanded meeting of the Scientific Problem Commission of the West Kazakhstan Marat Ospanov Medical University.

The results of the study were reported at scientific and practical conferences:

1) THE III INTERNATIONAL SCIENTIFIC AND EDUCATIONAL CONFERENCE // MINERVA MEDICA .

2) The IX Annual International Scientific-Practical Conference “Medicine Pressing Questions” May 6-8, 2020, Baku, AZERBAIJAN.

3) LVIX scientific conference of students and youth with international participation, dedicated to the 60th anniversary of the Student Scientific Society of West Kazakhstan

Marat Ospanov Medical University (April 24, 2019).
4) XIV International Scientific and Practical Conference of Young Medical Scientists // October, 2020.
5) International scientific and practical conference "MODERN MEDICINE: NEW APPROACH AND CURRENT RESEARCH" among medical educational organizations of Kazakhstan, near and far abroad, timed to the day of the World Day against Osteoporosis// Aktobe, 2021.

MATERIALS AND METHODS

General characteristics of the study.

The work was carried out at the Department of Obstetrics and Gynecology No. 2.

The clinical part of the study was carried out at the bases of city polyclinics and obstetric medical institutions in Aktobe city: all of them are public utility companies on the right of economic management (PUC on the REM): "City Polyclinic №1" address details: Aktobe city, Maresieva street 1B, "City Polyclinic №2" Aktobe city, Ahtanova street 50, " City Polyclinic №3" microregion #12. House №51, " City Polyclinic №4" Aktobe city, Br.Zhubanovyh 293B, PUC on the REM "City Polyclinic №5" Konaeva Street, "City Polyclinic №6" Aktobe city, Abulhaiyr-khan avenue 87, "Kargalinskaya city hospital" Aktobe city, Kargala region, Kurgulova street 19/B, "Aktobe regional consultative and diagnostic center " Aktobe city, Bogenbai batyr street 50, "Aktobe regional clinical hospital" Aktobe city, Zhanakonys region 8E, "Aktobe Regional Perinatal Center" Aktobe city, 3A.

The laboratory part of the study was carried out on the basis of the clinical laboratory of the PUC on the REM "Aktobe Regional Perinatal Center", Aktobe, Altynsarin street 3A, and the genetic part of the study was carried out on the basis of the scientific and practical center, Aktobe, Maresyev 74.

The work was carried out within the framework of the scientific and technical program "Placental growth factor-1 as screening for preeclampsia" state registration number 0119RKI0262, Department of Obstetrics and Gynecology No. 2, funded by the West Kazakhstan Marat Ospanov Medical University.

Study design:

1 stage – An observational prospective cohort study.
2 stage – Case control.

The object of the study is pregnant women. Sample size Stage 1 n = 304 people, Stage 2 n = 218 (case-101 and control-117), in the age range from 18 to 40 years. The genetic section of the study was conducted on only three generations of ethnically Kazakh women, because despite some progress in studying the molecular mechanisms of preeclampsia, the results of different researchers on the genetic predisposition to the disease often contradict each other. Therefore, taking into account ethnicity, it is important to study the structure of hereditary predisposition to preeclampsia and to look for general and ethno-genetic markers of this pathology.

The study does not recommend the use of measures that endanger the health of the object or fetus and does not recommend high or moderate levels of pain.

Study plan: Participants were selected by simple random sampling by means of random number generation, as a result of which study participants were identified according to the inclusion and exclusion criteria. Criteria for inclusion in the study:

In 1st stage:

1. Kazakh women.
2. Age from 18 to 40 years old.
3. Singleton pregnancy.
4. The gestation period is less than 14 weeks.
5. A living embryo without any abnormalities identified at this stage.
6. Voluntary informed consent of the patient to participate in the study.

In 2nd stage:

1. Kazakh women.
2. Age from 18 to 40 years old.
3. Singleton pregnancy.
4. Premature pregnancy.
5. A living embryo without any abnormalities identified at this stage.
6. Voluntary informed consent of the patient to participate in the study.

The criteria for exclusion from the study are:

At stage 1: 1. Other ethnic women. 2. Age less than 18 years old and over 40 years old. 3. Multiple pregnancy. 4. The gestation period is more than 14 weeks. 5. Chronic diseases: cardiovascular diseases, chronic kidney diseases, diabetes mellitus, autoimmune diseases, obesity (BMI> 30), thrombophilia, benign and malignant tumors. 6. Fetal abnormalities: intrauterine fetal malformations, suspected chromosomal abnormalities.

Stage 2:

1. Other ethnic women.
2. Age less than 18 years old and over 40 years old.
3. Multiple pregnancy.
4. Premature pregnancy.
5. Chronic diseases: cardiovascular diseases, chronic kidney diseases, diabetes mellitus, autoimmune diseases, obesity (BMI> 30), thrombophilia, benign and malignant tumors.
6. Fetal abnormalities: intrauterine fetal malformations, suspected chromosomal abnormalities.

Clinical part of the study.

Study was carried out in several stages.

1 stage - analysis of clinical and anamnestic indicators and data of gynecological examination, as well as blood sampling to determine the level of placental growth factor.

1. Survey. Collection of passport and ethnic data on three generations, past diseases, clarification of reproductive health and reproductive function, the course and outcomes of previous pregnancies, the course of this pregnancy, transfer of information to questionnaires.

2. General clinical examination: determination of anthropometric data (weight, height, BMI), systemic examination, special obstetric examination. Determination of the level of systolic and diastolic blood pressure by measuring it with a sphygmomanometer according to the standard method recommended by WHO.
3. Blood serum sampling in a volume of 5.0 ml. in one procedure, after an overnight fast, on an empty stomach, to determine the level of placental growth factor-1, by venipuncture by the method of vacuum sampling into AVATUBE vacutainers (EcoFarm International, Kazakhstan) in compliance with aseptic and antiseptic measures.
4. Collect urine into urine collection containers to quantify proteinuria.
5. Determination of the level of placental growth factor-1 in the blood by enzyme immunoassay using Human PIGF Quantitine ELISA Kit reagents.
6. Determination of the level of platelets in the blood as an additional method for diagnosing preeclampsia.
7. Determination of the level of transaminases ALT and AST as an additional method for diagnosing preeclampsia.
8. Fetal ultrasound to confirm or exclude intrauterine growth retardation: fetal fetometry - determination of the biparietal size and circumference of the fetal head, abdominal circumference and length of the femur, determination of the amniotic fluid index (AFI).

Then, before delivery, a prospective observation was carried out in order to establish the presence of preeclampsia.

A total of 218 women (101 cases and 117 observations) participated in the study "Situation-control" according to the 2nd stage of the study. The average age of the subjects was 29.0 (range 24.0-34.0) in group I and 27.0 (range 24.0-32.0) in group II. The weight and height index was 26.5 (3.0) in group I and 23.4 (3.4) in group II (<0.0001). In group I, 36% (n = 36) and 64% (n = 65) were employed, 42% (n = 49) and 58% (n = 58) were in group II, respectively. (p> 0.05) no statistical difference was detected. Heredity, ie 1 case of hypertension in group I, 2 cases in group II in the family history, and 1 case in group II in the family history of diabetes mellitus was not detected in the first group (p> 0.05). The median age of menarche was 13 years in both groups (<0.05). The median age of onset of sexual intercourse was 13 years in both groups and no statistical difference was found (p> 0.05). In the analysis of somatic and gynecological history of chronic extragenital diseases in group I pyelonephritis 3%, myopia 2%, and chronic arterial hypertension, rheumatoid arthritis, sinusitis 1 case, in group II myopia and mastopathy 2%, pyelonephritis 0.9% met. Varicose veins of the legs were detected in 6.9% in group I, 4.3% in group II (p> 0.05). In two groups of gynecological diseases, cervical erosion was 5.9% (n = 6) and 15% (n = 18).) in group I ovarian cysts and uterine fibroids in 1% of cases, respectively, in group II polycystic ovary syndrome and endometrial polyp in 1% of cases (p <0.05). The analysis of fertility parity did not reveal significant differences between groups (p> 0.05). The first births occurred in 42% of cases in group I and 27% of cases in group II. Repeated births were detected in 58% of cases in group I and 73% in group II. In the analysis of previous pregnancies, medical abortions were detected in 14% (n = 14) of group I subjects and 24% (n = 28) of group II subjects.

Medical abortions occurred in group I with a frequency of 20%, and in group II with a frequency of 17%.. In the analysis of the course of this pregnancy, the average value of GVA was 94 ± 15.0 (range 90–100) in group I, 68 ± 9.0 (range 60–70) mmHg in group II. The average value of SAR in group I was 155 ± 23.0 (range 140–170), in group II 108 ± 13.0 (range 100–110) mmHg. made up. The analysis of the course and outcome of labor revealed statistical differences between groups on premature / premature birth, which in turn may be due to premature birth (induction) in preeclampsia. At the same time, operative delivery was 36% in group I and 13% in group II. A statistical difference ($p < 0.05$) was found between the first group and the second group in assessing the weight and height of newborns and the condition of the newborn at 1 minute / 5 minutes on the Apgar scale. It should be noted that in the group of preeclampsia, stillbirths were detected in 2% of newborns. In the analysis of the distribution of the polymorphic variant of the placental growth factor (PLGF) rs 1042886 in the main and control groups, we analyzed the correspondence of the frequency of genotypes to the Hardy-Weinberg equilibrium, between the two groups . When analyzing the frequencies of empirical genotypes in groups and data on the transport of minor alleles, the incidence of genotypes in the group "Situation": GG 0.64%, GC 0.32%, CC 0.04%, in the group "Control" GG 0.67%, GC was detected in 0.30%, CC in 0.03%. Using one-factor logistic regression models to assess the relationship between anamnestic and clinical data with the risk of developing PE, a statistically significant relationship between the subject's age and menarche age, systolic blood pressure at admission to the maternity ward and diastolic blood pressure was identified. In order to develop a predictive model, a two-way selection of predictors was made on the basis of Akaike's information criteria. As a result of the assessment of the coefficients of the regression model obtained during the selection of the bilateral direction, only the menarche age, history of labor, systolic blood pressure at the time of admission to the maternity ward were included in the model to assess the likelihood of developing PE. When using the probability assessment of PE = 10% as a threshold value, the sensitivity of the model is 92.1 (range 85.0–96.5), the specificity is 89.5% [95% SI: 85.4; 92.8]. The relationship between preeclampsia and SNP rs1042886 polymorphism of the PlGF gene in Kazakh women has not been revealed.

CONCLUSION.

Based on the above results, the following conclusions can be made: In the first trimester of pregnancy, the average concentration of placental growth factor in the blood is 40.5 (24.9) pg / ml, the median is 35.5 (range 22.4–51.2) pg / ml, and The average concentration of placental growth factor in the urine was 27.7 (20.9) pg / ml, the median - 20.8 (range 13.8-34.6) pg / ml. In groups with preeclampsia, the median concentration of placental growth factor in the blood is 11.4 (range 8.6-33.0) pg / ml, the median concentration of placental growth factor in urine is 7.2 (range 5.8-18.0) pg. / ml was. In accordance with the task of determining the presence and frequency of SNP rs1042886 polymorphism of the PlGF gene in Kazakh women, data on the frequency of genotypes and minor allele transport were analyzed and the incidence of

genotypes in preeclampsia: GG 64%, GC 32%, CC 4% In the "control" group, GG was detected in 67%, GC in 30%, CC in 3%. In the presence of alleles in group I G was observed in 0.8, C in 0.2, in group II G in 0.18, C in 0.82. When analyzing the relationship between PLGF concentrations in the blood and urine using the risk of developing PE using one-factor logistic regression models, the probability of developing PE increases by an average of 1.54 times with each decrease in blood PLGF concentration by 10 pg / ml; When the concentration of PLGF in the urine decreases for every 10 pg / ml, the probability of developing PE increases by an average of 2.7 times. The AUC of PLGF in the blood is 0.74 [95% SI: 0.58; 0.90], for the concentration of PLGF in the urine - 0.79 [95% SI: 0.64; 0.93]. Analysis of the relationship between PLGF levels in the blood and urine with a sequential result formed as "uncomplicated GAG <mild PE <severe PE <severe eclampsia" revealed that a decrease in blood PLGF concentrations was associated with an increase in severity and risk of complications (every 10 pg / ml) OSH) was 0.68, 95% SI: 0.52; 0.84, p = 0.0001), and a decrease in the concentration of PLGF in the urine was associated with an increase in severity and risk of complications (every 10 pg / ml ratio (OC) 0.46, 95% SI: 0.29; 0.67, p <0.0001. One-factor logistics Using regression models, an assessment of the association of anamnestic and clinical data with the risk of developing PE revealed a statistically significant relationship to the age at which sexual intercourse began.

CONCLUSIONS OF SCIENTIFIC RESEARCH:

1. The obstetrician-gynecologist, in addition to the main diagnostic criteria for a comprehensive examination of pregnant women with preeclampsia, is recommended to determine the placental growth factor in the blood and urine.

2. According to the frequency of SNP rs1042886 PLGF gene polymorphism in Kazakh women in the group of preeclampsia GG 64%, GC 32%, CC 4%, in the group "Control" GG 67%, GC 30%, CC 3% of cases.

3. In Kazakh women in the first trimester of pregnancy with each decrease in the concentration of PLGF in the blood for every 10 pg / ml, the probability of developing PE increases by an average of 1.54 times; When the concentration of PLGF in the urine decreases for every 10 pg / ml, the probability of developing PE increases by an average of 2.7 times.
4. The relationship between preeclampsia and SNP rs1042886 polymorphism of the PLGF gene in Kazakh women has not been established.

PRACTICAL RECOMMENDATIONS

1. The obstetrician-gynecologist is recommended to determine the factor of placental growth in the blood and urine in addition to the main diagnostic criteria for a comprehensive examination of pregnant women with preeclampsia.

2. Information criteria for predicting the development of preeclampsia in the 1st trimester of pregnancy: - concentration of placental growth factor in the blood - less than 35.5 pg / ml; - Concentration of placental growth factor in urine - less than 20.8 pg / ml.
3. The concentration of PLGF in the urine can be used as a sensitive marker of the likelihood of developing preeclampsia than the concentration of PLGF in the blood.

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