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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE IMPORTANCE OF PLACENTAL GROWTH FACTOR AND CYTOKINES IN THE PROGNOSIS OF PREECLAMPSIA IN PREGNANT WOMEN

Speciality: 3215.01 – Obstetrics and gynecology

Field of science: Medicine

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Azərbaycan Tibb Universitetinin
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GENERAL CHARACTERISTICS OF WORK

The actuality of the subject. Widespread preeclampsia, multifactorial etiology and pathogenesis, inadequate effectiveness of treatment and prevention measures, high incidence of maternal and perinatal morbidity and mortality, deterioration of the health of a woman experiencing this complication of pregnancy, due to the high cost of intensive care in patients with this pathology are considered one of the most complex and unresolved problems of obstetrics in modern times.[1].Every year, 8.5 million cases of preeclampsia are registered in the world[2, 3]. Preeclampsia occurs in 2-8% of pregnancies and continues to be one of the leading causes of maternal and perinatal death[4]. High rates of fetal developmental delay in infants born to these mothers, premature birth, increased frequency of complications of the early neonatal period get attention[5,6].

Pregnancy during preeclampsia maintains a leading position in the structure of pathologies, which is explained by the lack of early and

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[5] *Алафинова, Ю.А.* Досрочное родоразрешение и перинатальные исходы беременности, осложненной преэклампсией. Нарушение маточно-плацентарного кровотока при преэклампсии / Ю.А.Алафинова, Е.В.Говорунова, А.А.Сверчинская [и др.] //Аллея науки, – 2017. Т. 1, – № 16, – с. 474-476.

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accurate diagnostic criteria, more effective prevention and treatment measures. Despite the great attention paid to this problem all over the world, untill now certain issues of its pathogenesis, prognosis, search for accurate diagnostic markers, prevention and, consequently, pathogenetically justified treatment of this dangerous complication of pregnancy remain unresolved.

In modern times, preeclampsia is called a "theoretical disease" because about 30 theories are known about its development.Today, there is no theory that fully explains the origin of preeclampsia. It has been shown that the source of complications of the gestation process is a violation of trophoblast invasion, vasculogenesis and angiogenesis [7] .Violation of these stages can lead to incomplete invasion of endovascular trophoblasts in the spiral uterine arteries, which affects the structure and function of the placenta and leads to and incomplete pregnancy, preeclampsia and fetal developmental delay. Factors that cause widespread endothelial dysfunction and systemic inflammatory response syndrome as a result of abnormal placentation and perfusion in the placenta are eliminated, which also leads to polyorganic insufficiency.

In recent years, the role of angiogenic growth factors in the pathophysiology of various obstetric pathologies, including preeclampsia, is increasingly being discussed[8]. During preeclampsia, changes in the ratio of angiogenic growth factors are observed, which lead to disruption of normal angiogenesis processes and endothelial repair during pregnancy. Many growth factors have been described in modern times, most of which affect the function of the female reproductive system.

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[8]*Chaiworapongsa, T., Romero, R., Erez, O.* The prediction of fetal death with a simple maternal blood test at 24-28 weeks: a role for angiogenic index-1 (PLGF/SVEGFR-1 ratio) //American Journal of Obstetrics and Gynecology, – 2017. 6, – p. 682.

Placental growth factor (PlGF) is of particular interest - it is considered an important marker of angiogenesis[9,10,11].

Measurement of serum PlGF (placental groth factor) is extremely important for the diagnosis of preeclampsia. Given that the clinical symptoms of preeclampsia are not always informative and the accuracy of the diagnosis is often in doubt, the determination of PlGF may be of great practical importance.Cytokines, which are the main mediators of the interaction of the cells of the immune system of the mother and fetus, are very important for the physiological course of pregnancy, as they play an important role in implantation, embryo growth and development[12].Cytokines distinguish between anti-inflammatory and anti-inflammatory regulators of cellular and humoral immunity.Cytokines are actively involved in the formation of the mother's immune response and liaise between the body's nervous, immune and endocrine systems. A comprehensive study of PlGF and cytokines allows a deeper understanding of the processes occurring in the "mother-placenta-fetus" system during physiological pregnancy, as well as during preeclampsia, which is a complication of the gestation process.

So in modern times, obstetricians and gynecologists face a issue ofsearch for new diagnostic criteria that allow early detection of patients at high risk of developing preeclampsia, early detection of its symptoms and prognosis of obstetric tactics. Predicting the development of preeclampsia that based on early complex determination of placental growth factors and cytokines in peripheral blood has determined the relevance of the current research.

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[9]*Chau K., Hennessy A., Makris A.* Placental growth factor and preeclampsia //J. Hum Hypertens., – 2017. 31, – p. 782-786.

[10]*Kleiner, J.* Activator protein-1 contributes to the nacl-induced expression of VEGF and PLGF IN RPE cells / J.Kleiner, M.Hollborn, P.Wiedemann [et al.] //Molecular Vision, – 2018. 24, – p. 647-666.

[11]*Makris, A., Yeung, K. R., Lim, S.M.* Placental growth factor reduces blood pressure in a uteroplacental ischemia model of preeclampsia in nonhuman primates // Hypertension, – 2016. 67 (6), – p. 1263-1272.

[12]*Радьков, О.В.* Биомаркеры прогнозирования и диагностики преэклампсии // Acta Biomedica Scientifica, – 2018. Т. 3. № 2, –с. 20-24.

Object of research. 120 pregnant women (90 women with preeclampsia and 30 women without preeclampsia).

The aim of the study was to determine the clinical significance of placental growth factors and clinical examination of blood cytokines as biomarkers of the development of preeclampsia of varying severity.

Research objectives:

1. Carry out examination of clinical-anamnestic and laboratory indicators of pregnant women and to identify risk factors for the development of preeclampsia of varying severity.
2. To study the dynamics of PlGF placental growth factor levels in the blood serum of healthy pregnant women and patients having preeclampsia of varying severity.
3. To study the characteristics of the expression of inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines in the blood serum of pregnant women in the 10-14 and 20-22 weeks of pregnancy as predictors of the development of preeclampsia.
4. To study and make a comparative analysis of the relationship between cytokine levels and placental growth factor during physiological and complicated pregnancy.
5. Develop an algorithm for individual prediction of the risk of developing preeclampsia in early pregnancy.

Research methods. Clinical-anamnestic and laboratory research methods.

The main provisions of the dissertation which presented defense:

- Pregnant women with preeclampsia are characterized by imbalances between proangiogenic and antiangiogenic factors.
- Levels of PlGF below 45 pg/ml at 10-14 weeks are of greater diagnostic value during the development of a severe form of preeclampsia (OR = 3.28±0.27; sensitivity - 76%; specificity - 63%).The diagnostic value of PlGF shifts to mild preeclampsia during 20-22 weeks of gestation (OR = 3.0±0.41; sensitivity - 75%; specificity - 54%).

- Hyperproduction of inflammatory cytokines and insufficient entry of anti-inflammatory cytokines into the bloodstream aggravates the dangerous course of preeclampsia in the future development of pregnancy.
- Changes in clinical-anamnestic, hematological, hemostasiological and biochemical parameters should be taken into account when assessing the severity of preeclampsia.

Scientific novelty of the research.

- In Azerbaijan, a comprehensive study of the level of PlGF placental growth factor, pre-inflammatory and anti-inflammatory cytokines in the blood of pregnant women with preeclampsia was performed and their prognostic significance was determined.
- The correlation dependence occurring between PlGF placental growth factor and cytokines, which characterizes the course of pregnancy in the normal and during the development of preeclampsia, was determined.
- Diagnostic significance of PlGF concentration changes in the mother's blood at 10-14 and 20-22 weeks of pregnancy, during mild and severe preeclampsia has been shown.
- In Azerbaijan, the following pathogenetic factors have been identified to predict the individual risk of developing preeclampsia: for severe preeclampsia, BMI $\geq$25 kg/m^2 and PlGF <45 pg/ml (10-14 weeks), history of pyelonephritis for mild preeclampsia and PlGF <45 pg/ml (20-22 weeks).

Practical significance of the research.

- Determination of PlGF placental growth factor allows to predict the development of preeclampsia in early pregnancy until the onset of the first clinical and laboratory symptoms.
- Assignment of inflammatory and anti-inflammatory cytokines in the early stages of gestation is consided a promising immunological marker of the risk of developing severe preeclampsia.

- Complex determination of PlGF placental growth factor and blood cytokines should be used in the laboratory diagnosis of preeclampsia.
- An algorithm for individual prediction of the risk of developing preeclampsia was developed based on a combination of more important clinical-anamnestic and angiogenic factors during the examination of pregnant women at 10-14 and 20-22 weeks of pregnancy which will allow the formation of risk groups for the development of preeclampsia in early pregnancy.

Approbation of the case. The main provisions of the dissertation "Современная медицина: новые подходы и актуальные исследования" were presented at the XXVII-XXVIII international conference (Moscow, 2019), at the international conference dedicated to the 100th anniversary of the Department of Human Anatomy and Medical Terminology of the Azerbaijan Medical University (Baku, 2019).The materials of the dissertation were reported and discussed at the meeting of the II Department of Obstetrics and Gynecology of Azerbaijan Medical University (AMU) (11.02.2019, protocol № 01), at the scientific seminar of the Dissertation Council ED 2.06 (29.04.2022, protocol № 14).

Where the work is done. The dissertation work was carried out at the II Department of Obstetrics and Gynecology of AMU.

Application of research results in practice. The results of the research were included in the teaching process of the II Department of Obstetrics and Gynecology of AMU, as well as in the clinical practice of the Teaching Surgery Clinic of AMU.

Publications: 10 scientific works on the topic of the dissertation have been published. 6 of them are articles, 4 theses, including 3 articles and 2 theses were published in foreign publishing houses.

The structure and scope of the dissertation. The dissertation work consists of 141 (172075 characters) and introduction (5 pages), literature review (29 pages), materials and methods chapter (8 pages), results of personal researches from 3 chapter (57 pages), conclusion (12 pages), results (2 pages), practical recommendations (1 page) and bibliography (24 pages).

The dissertation work is illustrated with 32 tables, 14 graphs, 1 picture and 1 scheme. The list of literature includes 217 sources, 11 of which are in Azerbaijani, 104 in Russian and 102 in English.

MATERIALS AND METHODS OF RESEARCH

90 pregnant women with mild and severe preeclampsia underwent a comprehensive clinical and laboratory examination. Patients received initial consultative and diagnostic assistance, were observed in the dynamics of the gestation process, underwent the necessary examinations, received treatment, and were prepared for childbirth.

The diagnosis of preeclampsia during pregnancy was made on the basis of patient complaints, clinical examination, somatic, obstetric and gynecological anamnesis, analysis of the current pregnancy, ultrasound fetometry and dopplerometry. Diagnosis is formed in accordance with the 10th Revision of International Classification of Diseases and Related Health Problems (ICD-10). The severity of preeclampsia was assessed in accordance with the normative documents and criteria regulated by the Ministry of Health of the Republic of Azerbaijan.

A woman with a history of moderate preeclampsia with normal AT has arterial hypertension that occurs at> 20 weeks of pregnancy: it is indicated when SAT ≥ 140 mm Hg or DAT ≥ 90 mm Hg and proteinuria ≥ 0.3 g / l protein is detected in the daily urine sample. Severe preeclampsia is diagnosed when one or more of the following symptoms are present: AT 160/100 mm Hg and more should be measured at least 2 times at 6 hour intervals in the horizontal position of the pregnant woman;proteinuria 5 g / day protein and more or 3+ in separate portions; oliguria - 500 ml or less of urine per day;pains under the epigastric or right rib; pulmonary edema or pulmonary insufficiency; liver dysfunction;neurological symptoms thrombocytopenia; according to US, fetal growth retardation at 4 weeks and longer.

Retrospective and prospective approaches to obtaining initial data were used in the study. During retrospective examinations, the information was obtained by selecting and collecting medical records

from pregnant women. Questionnaires were developed for prospective examinations which included all the information obtained in the course of the research (complaints, social and occupational factors, information on obstetric and gynecological anamnesis, information on clinical-laboratory, instrumental examination methods, on the outcome of pregnancy). Information about the patients participating in the study was entered into a personal computer database.

2 main examination groups were formed on the basis of the duration of gestation, bstetric and gynecological anamnesis, as well as clinical-laboratory and functional data. The first group consisted of 76 (84.4%) patients with moderate preeclampsia (average age - 26.2±0.59 years). The second group consisted of 14 (15.6%) women with severe preeclampsia (mean age -29.1±1.32 years). The control group consisted of 30 women with a physiological course of pregnancy and an uncomplicated obstetric and gynecological history. The mean age of pregnant women in this group was 26.4±0.66 years.

Criteria for inclusion in the study: duration of gestation 34-38 weeks; verified preeclampsia diagnosis; single fetus pregnancy; spontaneous pregnancy.Criteria for exclusion: multiple pregnancies; pregnancy ocurring as a result of assisted reproductive technologies; chromosomal abnormalities and congenital malformations of the fetus; severe extragenital pathology and chronic diseases in the stage of decompensation in pregnant women;acute infectious diseases. Absence of markers of obstetric pathology was taken into account during hormonal, clinical, ultrasound, dopplerometric and biochemical examination as the criteria for inclusion in the control group.

The risk factors for developing preeclampsia in all examined women were assessed, and the characteristics of family and personal anamnesis were studied in detail.Study of a woman's age at the time of pregnancy, body weight index in dynamics, infectious diseases, presence of acute and chronic somatic pathology, bad habits, study of issues such as taking medication for 1 month before pregnancy and throughout pregnancy include to the analysis of general and obstetric history. During the collection of gynecological anamnesis, special

attention was paid to menstrual dysfunction, specific and non-specific inflammatory diseases of the reproductive system (STIs), infertility, operative and intrauterine interventions. During the external obstetric examination, the condition, position, arrival of the fetus, the nature of its motor activity were determined, the frequency of fetal heartbeats was determined, the circumference of the abdomen and the height of the bottom of the uterus were measured, it was also determined that the size of the uterus corresponds to the duration of gestation.

In all patients with preeclampsia, BMI was calculated taking into account height and weight, which was calculated as the ratio of body weight to square digits according to the following formula: BMI = body weight (kg) / height (m^2).

All patients underwent standard examinations: determination of blood group and rhesus factor, clinical and biochemical examinations of blood, hemostasiogram, general examination of urine, determination of daily proteinuria, examination of the uterine smear and bacteriological examination of secretions.

The study of peripheral blood was performed using a hemolytic analyzer in accordance with the requirements of clinical and hematological examination. Hemostasiological examination includes determination of clotting time, prothrombin index, fibrinogen, activated partial thromboplastin time (ARTT) and thrombin time (TT). Determination of total protein, residual nitrogen, urea, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) in blood serum was performed in a biochemical analyzer using standard computer programs and reagents. Determination of placental height factor (PlGF) was performed at 10–14 and 20–22 weeks of gestation during pregnancy.The tests were performed on the AlereTriage analyzer (Alere, USA) by immunofluorescence analysis, strictly following the manufacturer's recommendations.Serum levels of IL-6, TNF-α and IL-10 cytokines were carried out during pregnancy at 10–14 and 20–22 weeks of gestation in a semi-automated analyzer with enzyme-linked immunosorbent assay (MS BioScreen 500, USA) using a system set (ZAO Vector-Best, Russia).

Methods of statistical processing. The data of the study were processed on a personal computer using modern software - Microsoft Excel spreadsheet editor and IBM SPSS Statistics 22 computer program for processing statistical data.The data obtained through questionnaires, as well as information on clinical and laboratory examination methods were collected in the Excel database.To characterize a group of homogeneous units, their average mathematical values (M) and standard errors (m) were determined.When comparing data between groups obtained in the course of the study, 2 methods of estimating the differences of independent samples - Student's t-criterion and Fisher's exact method were applied.The correlation analysis was performed using Pearson's linear correlation coefficient. To assess the relative risk, connection tables were analyzed: odds ratio (OR) and two-sided 95% confidence intervals (CI).

Calculations were made according to the following formula:

$$OR = [A \times D] / [B \times C],$$

where A and B are the presence of the symptom in patients in the main and control groups, and C and D are its absence in these groups, respectively.

Statistical analysis of the specificity (Sp), sensitivity (Se) and likelihood ratio (LR) of the prognostic test was carried out by compiling 4-strip tables. An accuracy level of $p<0.05$ was considered statistically significant for all types of analysis.

RESULTS OF RESEARCH AND THEIR DISCUSSION

To identify risk factors for the development of preeclampsia and its severity, we conducted a comparative assessment of clinical and paraclinical features in 120 patients and they are divided into basic and control groups. The main group included 90 (75.0%) patients with preeclampsia based on clinical and laboratory signs. The control group included 30 (25.0%) women with uncomplicated obstetric and gynecological history and physiological course of pregnancy (without preeclampsia).

Pregnant women those have preeclampsia were divided into 2 groups. The first group included 76 (84.4%) patients with moderate preeclampsia, and the second group included 14 (15.6%) women with severe preeclampsia.Patients with moderate to severe preeclampsia were compared by age:age 26.2±0.59 years and 29.1±1.32 years ($p>0.05$), respectively. In the first and second groups of patients, the course of pregnancy was aggravated by preeclampsia at 34 weeks and later.The gestation period in the group was 34-38 weeks. The age composition of women in the groups is approximately the same. The majority of patients were under 30 years of age, and there were no significant differences in age distribution. Analysis of family anamnesis shows that more than half of women having preeclampsia - 68.9% were married by imam (religious marriage). During the study of social factors, it was found that in the main group, 52.2% of women are housewives, 12.2% are students, and 35.6% of patients are working women. Thus, a comparison of these indicators, ie age, education, family and social status, showed that the different groups of women were homogeneous.

Metabolic disorders are important in terms of the risk of developing preeclampsia. Pregnant women with preeclampsia are characterized by high BMI and presence of clinically significant obesity is typical. In the first trimester, the body mass index (BMI) was significantly higher in women with preeclampsia than in pregnant women with a physiological course (23.1 [17.1-31.1] kg/m^2; in exchange for 26.4 [16.2-42.8] kg/m^2; $p = 0.0001$). Body mass index was 26.2 [16.2-42.8] kg/m^2 ($p = 0.0002$) in women with moderate preeclampsia and 27.4 [17.3-37.5] kg/m^2 in pregnant women with severe preeclampsia. ($p = 0.015$). In the group of women with severe preeclampsia, overweight and obesity 71.4% (n=10) were registered in pregnant women, in the group with mild preeclampsia - 52.6% (n=40) of pregnant women,in the control group - 26.7% (n=8) were registered in pregnant women ($p<0.05$).

Extragenital pathologies have a great impact on the female body before occurance of pregnancy and during the entire gestation period,

affect the formation of the couple, increase perinatal morbidity and mortality, by complicating the course of pregnancy and childbirth.

In patients whose pregnancy aggravated with preeclampsia regardless of severity, significantly more were found in the control group than in women: pyelonephritis (36.7%), anemia (50.0%), arterial hypertension (21.1%). Anemia in pregnant women was found in half of the women in the main group, which is 3.0 times higher than in the control group - 16.7% ($p<0.05$).

During the collection of obstetric and gynecological anamnesis data, it was found that the duration of the menstrual cycle in patients with preeclampsia was 21-24 days in 30.0% of women ($p<0.05$). The duration of the menstrual cycle was 25-30 days in 55.6% of women.The duration of the menstrual cycle was 31-35 days and was observed in 14.4% of women with preeclampsia.

In the anamnesis of the main group of women, the mean age of onset of menarche was 13.4±0.13 years, in the control group - 13.2±0.21 years.When reviewing the gynecological anamnesis of women in the main group, the high frequency of various disorders of the menstrual cycle in 36.7% (n=33) women is noteworthy;Chronic inflammatory diseases of the small pelvic organs and ovarian dysfunction were found in 5.6% (n=5) of women, erosion of the cervix in 6.7% (n=6).

The data obtained showed that obstetric complications were more common in the main group. In this regard, relative risk indicators for pregnancy complications and comorbid conditions have been calculated. Thus, preeclampsia in 6.7% (n=6) patients aggravated the risk of of pregnancy disorders.

Relative risk: OR = 1.35±0.05 (95% CI: 1.21–1.51; $p<0.05$). Preeclampsia ocurred with edema in 11.1% (n=10) of patients. Relative risk: OR = 1.24±0.11 (95% CI: 0.99-1.54; $p<0.05$). In the main group, 11.1% (n=10) patients had rhesus conflict. Relative risk was: OR = 1.03±0.16 (95% CI: 0.74–1.41; $p>0.05$). Preeclampsia in 3.3% (n=3) pregnant women was associated with antiphospholipid syndrome. Relative risk was: OR = 1.34±0.05 (95% CI: 1.20-1.49; $p<0.05$). The placental arrival incidence was found in 2.2% (n=2) of

patients with preeclampsia. Relative risk was: OR = 1.34±0.05 (95% CI: 1.20-1.49; p<0.05).

Caesarean section was performed in 44 (57.9%) women with mild preeclampsia and in 14 (100%) patients with severe preeclampsia (p<0.001), taking into account the aggravated course of pregnancy and the need for premature birth of patients in the main group. Relative risk was: OR = 1.94±0.12 (95% CI: 1.52–2.46; p<0.05, respectively) and OR = 8.50±0.47 (95% CI: 3.38-21.34; p<0.001).

The analysis of prognostic factors for the development of moderate and severe preeclampsia was based on the calculation of relative risk and 95% reliability interval. There is a link between age and the likelihood of developing preeclampsia in patients over 30 years of age. The relative risk of developing preeclampsia in women over 30 years of age was: OR = 1.06±0.11 (95% CI: 0.85-1.32; p> 0.05). In the anamnesis, preeclampsia was registered in 20 (22.3%) patients in the main group. Therefore, the relative risk of developing preeclampsia during the current pregnancy was: OR = 1.43±0.06 (95% CI: 1.25-1.62; p<0.05). Preeclampsia, which is a complication of pregnancy, is clinically characterized by arterial hypertension. The relative risk of developing preeclampsia in patients with arterial hypertension was: OR = 1.42±0.06 (95% CI: 1.25-1.61; p<0.05). The relative risk of developing preeclampsia in patients with pyelonephritis was: OR = 1.52±0.08 (95% CI: 1.31-1.77; p<0.05). The relative risk of developing preeclampsia in patients with anemia was: OR = 1.40±0.10 (95% CI: 1.14-1.70; p<0.05). The relative risk of developing preeclampsia in overweight patients was: OR = 1.33±0.10 (95% CI: 1.08-1.65; p<0.05). Various menstrual dysfunctions were reported in 33 (36.7%) pregnant women with preeclampsia. The relative risk of developing functional menstrual disorders in women with preeclampsia was: OR = 1.52±0.07 (95% CI: 1.31-1.77; p<0.05). The relative risk of developing preeclampsia in first-born women was: OR = 1.0±0.10 (95% CI: 0.81-1.23; p>0.05). The relative risk of developing preeclampsia in patients having abortion in the anamnesis was: OR = 0.76±0.17 (95% CI: 0.54-1.06; p> 0.05). The relative risk of developing preeclampsia in

patients with a history of incomplete pregnancy was: OR = 1.44±0.06 (95% CI: 1.26-1.64; p <0.05).

According to the estimates of the relative risk indicator, the prognostic factors for the development of preeclampsia are divided into weak and strong. Values with weak factors OR <2 were accepted. Factors with OR> 2 values were strongly accepted. The calculation of relative risk indicators allows to identify prognostic factors in the development of preeclampsia, taking into account the importance of each of them. The presence of preeclampsia and incomplete pregnancy in the anamnesis increases the risk of developing its severity during the current pregnancy by 3 and 4 times, respectively. During the current pregnancy, menstrual dysfunction and arterial hypertension increase the risk of developing severe preeclampsia by 4 times, pyelonephritis and anemia by 3 times. A pregnant woman over the age of 30 increases the risk of developing severe preeclampsia by 1.8 times.

In order to determine the role of laboratory factors in the pathogenesis of preeclampsia, quantitative measurements of PlGF, IL-6, TNF-α and IL-10 in the blood serum of pregnant women at 10-14 and 20-22 weeks of pregnancy were determined.

The distribution of values of the determined PlGF level in all pregnant women with preeclampsia corresponded to the median 45 pg/ml. To assess the relationship between PlGF levels and the prognosis of moderate to severe preeclampsia, marker levels in blood serum of all pregnant women in the main group were divided into two subgroups above and below the distribution median: > 45 pg/ml and <45 pg/ml, respectively. Estimation of relative developmental risk (NR) of moderate and severe preeclampsia was calculated for the low starting level of PlGF

Among all patients with preeclampsia, 46 (51.1%) pregnant women had PLGF levels below 45 pg/ml at 10-14 weeks of gestation. The risk of developing severe preeclampsia in these patients is 3.3 times higher than in other pregnant women (n=44) (NR = 3.28±0.27; 95% CI: 1.94-5.53; sensitivity - 76%; specificity - Was 63%), which was confirmed by the presence of severe preeclampsia in 14 (30.4%) patients.

The risk of developing moderate preeclampsia was 1.4 times higher (NR = 1.43±0.17; 95% CI: 1.01-2.02; sensitivity - 59%; specificity - 56%) in 46 (51.1%) patients with PLGF levels below (<45 pg/ml) the threshold than in other pregnant women (n=44), which it was confirmed by the development of moderate preeclampsia in 32 (69.6%) patients.

PlGF levels were below 45 pg/ml in 21 (23.3%) of all patients with preeclampsia at 20-22 weeks of gestation. The risk of developing severe preeclampsia in these patients is 1.5 times higher than in other pregnant women (n=69) (OR = 1.5±0.31; 95% CI: 0.81-2.76; sensitivity - 60%; specificity - 52%) which it was confirmed by the development of severe preeclampsia in 14 (66.7%) patients.

The risk of developing moderate preeclampsia was 3.0 times higher (NR = 3.0±0.41; 95% CI: 1.34-6.70; sensitivity - 75%; specificity - 54%) in 21 (23.3%) pregnant women with PlGF levels below the threshold (<45 pg/ml) than in other pregnant women (n=69), which was later confirmed by the development of moderate preeclampsia in 7 (33.3%) women.

Thus, the level of PlGF in the main group of patients ranged from 6.8 to 86.3 pg/ml. During 10-14 weeks of gestation, the mean level of PlGF in the blood serum in pregnant women with mild preeclampsia was 2.5 times lower than in pregnant women in the control group (42.6±1.33 pg/ml and 108.3±8.24 pg/ml, respectively, p<0.001). During 20-22 weeks of gestation, mean PlGF levels in the blood serum were slightly elevated to 61.7±1.52 pg/ml, but in the control group (222.2±22.64 pg/ml, p<0.001) was 3.6 times less than the same indicator. During 10-14 weeks of gestation, the mean level of PlGF in the blood serum of pregnant women with severe preeclampsia was 13.4 times lower than that of pregnant women in the control group (8.1±0.26 pg/ml and 108.3±8.24 pg/ml, respectively, p<0.001) and 5.3 times lower than pregnant women those have mild preeclampsia (8.1±0.26 pg/ml and 42.6±1.33 pg/ml, respectively, p<0.001). During 20-22 weeks of gestation, the mean level of PlGF in the blood serum was slightly elevated and was 11.3±0.52 pg/ml, but was 20 times lower than the same indicator in the control group (222.2±22.64 pg/ml, p<0.001).and 5.5 times less

than pregnant women those have mild preeclampsia (61.7±1.52 pg/ml, p<0.001) (Table 1).

Table 1

The amount of placental growth factor in the blood serum of examined patients

Duration of hestation	Main group			Control group (n=30)
	Mild preeclamp-sia (n=76)	Severe preeclampsia (n=14)	Preeclampsia total group (n=90)	
10-14 weeks	42,6±1,33 (8,1 - 66,9) p<0,001	8,1±0,26 (6,8 - 9,6) p<0,001 p1<0,001	37,2±1,73 (6,8 - 66,9) p<0,001	108,3±8,24 (33,6 - 216,5)
20-22 weeks	61,7±1,52 (11,9 - 86,3) p<0,001	11,3±0,65 (8,9 - 18,1) p<0,001 p1<0,001	53,9±2,32 (8,9 - 86,3) p<0,001	222,2±22,64 (96,7 - 642,5)

Note: p - the differences with respect to the control group are significant; p1 – in relation to severe preeclampsia

Thus, the measurement of PlGF levels in pregnant women with preeclampsia is of prognostic importance in the development of moderate and severe forms of it at different stages of hestasis.

The advantage of PlGF is that the concentration of this growth factor reflects the processes of placentation, angiogenesis, invasion of cytotrophoblasts into the spiral arteries of the mother and its amount can always be measured in the early stages of hestation.

The data of our study confirm that decreased levels of PlGF in the blood serum of pregnant women are considered an early marker of the development of preeclampsia and confirms the possibility of its development. The lower this value, the preeclampsia will be the more severe. These stages of pregnancy selected for the study are conditioned by the fact that at this time the planned prenatal diagnosis is carried out. Therefore, assessing the risk of developing

preeclampsia during these stages of pregnancy may lead to a reduction in the frequency of complications and perinatal losses. Thus, the adoption of appropriate measures in some cases allows you to timely correct the pathological condition and resolve the timing of implementation of delivery (labour).

Examination of serum IL-6 cytokine concentrations showed that in the main group of pregnant women at the beginning (at 10-14 weeks) its average amount was 52.0±0.56 pg/ml (range of change was 45.5-69.1 pg/ml).), which was 6.4 times higher than the value in the control group (8.1±0.30pg/ml, $p<0.001$). In the second phase of the study (20–22 weeks), the level of IL-6 was 54.4±0.49 pg/ml (range of change was 45.5–69.1 pg/ml) which was 5.7 times higher than the norm and 1.1 times higher than the initial level.

Determination of TNF-α showed that the level of TNF-α in pregnant women in the main group at 10-14 weeks of gestation was 58.7±0.80 pg/ml (range of change 49.3–78.3 pg/ml) which was 2.1 times higher than in the control group (27.5±0.53 pg/ml, $p<0.001$). During re-examination at 20-22 weeks, TNF-α levels in the blood serum were 61.3±0.74 pg/ml (range 52.1 to 79.9 pg/ml), which was 1.8 times higher than the normative values (29.0±0.52pg/ml, $p<0.001$). Analysis of the dynamics of changes in the anti-inflammatory cytokine IL-10 showed that, the mean level in pregnant women in the main group for 10-14 weeks was 6.6±0.07pg/ml (range of change 5.1 - 8.1pg/ml), which is higher than the values in the control group (9.2±0.16, $p<0.001$) was 1.4 times lower. During re-examination at 20-22 weeks, IL-10 levels in the serum blood were 6.4±0.07 pg/ml (range of 5.0 to 7.6 pg/ml), which was also 1.2 times lower than the control values (7.8±0.11pg/ml, $p<0.001$). Depending on the duration of gestation and the severity of the preeclampsia, a comparison of serum cytokine levels in pregnant women revealed that significantly higher levels of TNF-α cytokines are observed at 10-14 weeks of gestation during severe preeclampsia (Table 2, Fig. 1, 2).

Table 2

Serum cytokine levels at 10-14 and 20-22 weeks of gestation
M±m (min - max)

Indicators	Main group (n=90)			Control group (n=30)
	Mild preeclampsia (n=76)	Severe preeclampsia (n=14)	Preeclampsia total group (n=90)	
10-14 weeks				
IL-6, pg/ml	50,1±0,33 (45,5 - 59,9) p<0,001	62,3±0,80 (58,3 - 69,1) p<0,001 p_1<0,001	52,0±0,56 (45,5 - 69,1) p<0,001	8,1±0,30 (5,3 - 10,3)
TNF-α, pg/ml	55,8±0,40 (49,3 - 64,3) p<0,001	74,5±0,68 (71,8 - 78,3) p<0,001 p_1<0,001	58,7±0,80 (49,3 - 78,3) p<0,001	27,5±0,53 (20,6- 32,1)
IL-10, pg/ml	6,8±0,05 (6,1 - 8,1) p<0,001	5,4±0,08 (5,1 - 5,8) p<0,001 p_1<0,001	6,6±0,07 (5,1 - 8,1) p<0,001	9,2±0,16 (7,9 - 11,0)
20-22 weeks				
IL-6, pg/ml	52,7±0,27 (42,7 - 57,3) p<0,001	63,9±0,43 (62,2 - 67,2) p<0,001 p_1<0,001	54,4±0,49 (42,7 - 67,2) p<0,001	9,4±0,29 (6,4 - 12,5)
TNF-α, pg/ml	58,7±0,43 (52,1 - 67,0) p<0,001	75,0±1,11 (66,2 - 79,9) p<0,001 p_1<0,001	61,3±0,74 (52,1 - 79,9) p<0,001	29,0±0,52 (21,1 - 33,8)
IL-10, pg/ml	6,6±0,05 (6,0 - 7,6) p<0,001	5,3±0,08 (5,0 - 5,8) p<0,001 p_1<0,001	6,4±0,07 (5,0 - 7,6) p<0,001	7,8±0,11 (6,6 - 9,2)

Note: p - relative to the control group; p_1 - the differences are significant compared to severe preeclampsia

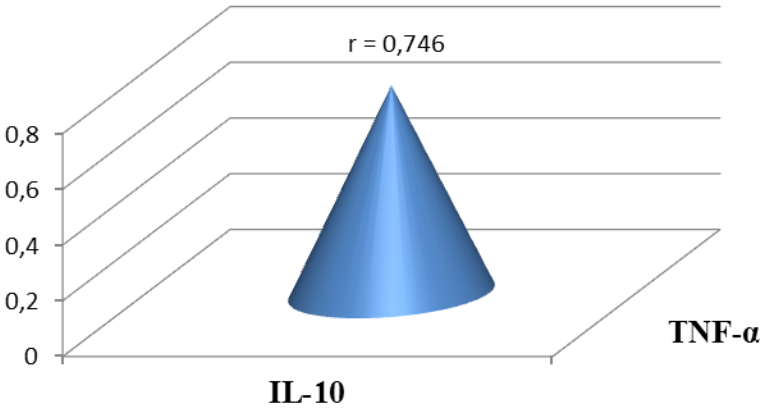

Fig. 1. Correlation between IL-10 and TNF-α in the blood serum of pregnant women with severe preeclampsia (10-14 weeks)

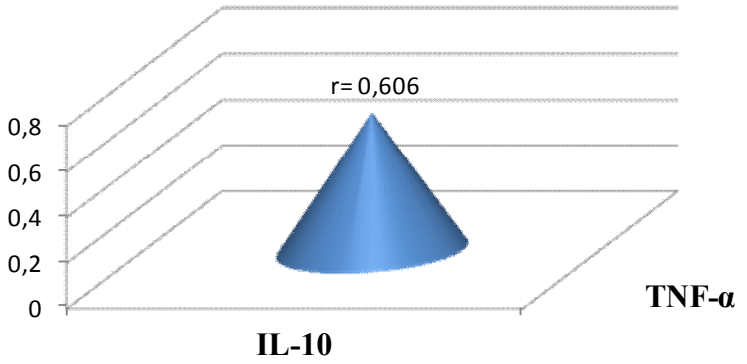

Fig. 2. Correlation between IL-10 and TNF-α in the serum of pregnant women with severe preeclampsia (20-22 weeks)

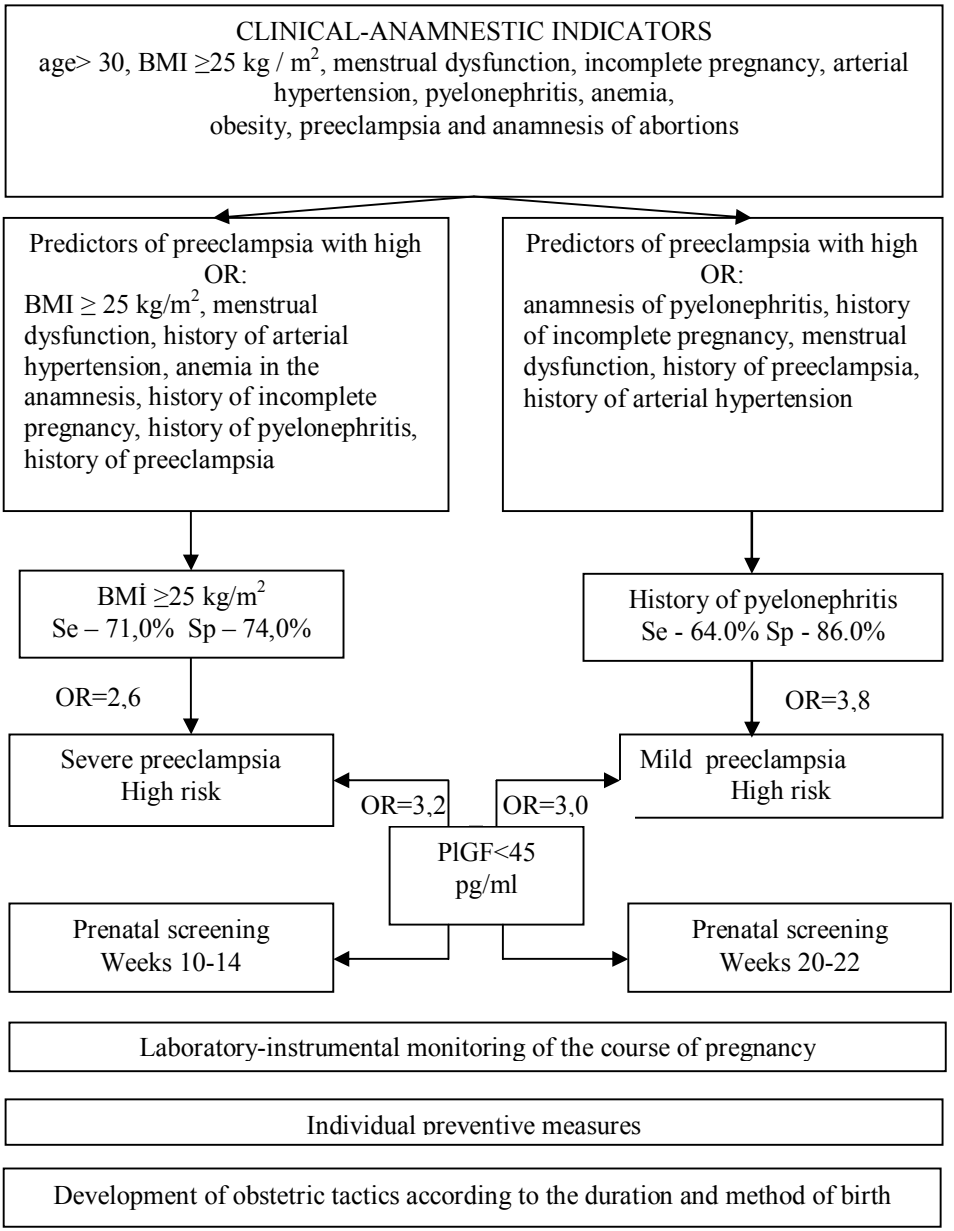

Fig. 3. Algorithm for individual prediction of the risk of developing preeclampsia

Depending on the severity of preeclampsia, the sensitivity (Se) and specificity (Sp) of the detected clinical-anamnestic parameters involved in predicting the development of mild and severe preeclampsia were assessed. Analyzing all risk factors, an aggregate of more important clinical-anamnestic and angiogenic factors was found during the mandatory examination of pregnant women by us at 10-14 and 20-22 weeks of pregnancy.

Influenced the formation of pathogenetically significant clinical and anamnestic indicators and angiogenic factors for preeclampsia in pregnant women: - BMI≥25 kg/m^2 and PlGF <45 pg/ml (10-14 weeks) during severe preeclampsia,during mild preeclampsia anamnesis of pyelonephritis and PlGF <45 pg/ml (20-22 weeks) (Figure 3).This prediction algorithm allows the formation of risk groups on the development of preeclampsia and its clinical course in early pregnancy and creates conditions for more effective implementation of treatment and prevention measures on the prevention of this pathology of pregnancy.

RESULTS

1. Significant risk factors for the development of moderate preeclampsia are: pyelonephritis 64.3% (OR = 3.85) in the anamnesis, incomplete pregnancy 40.8% (OR = 2.69) in the anamnesis, menstrual dysfunction 36.8% (OR = 2.58), preeclampsia 23.7 % (OR = 2.31) in the anamnesis, arterial hypertension 13.2% (OR = 2.16) in the anamnesis. Risk factors for the development of severe preeclampsia are:BMİ ≥ 25 kg/m^2 71.4% (OR = 2.66), menstrual dysfunction 35.7% (OR = 2.56), arterial hypertension in the anamnesis28.5% (OR = 2.40), anemia in the anamnesis 57.1% (OR = 2.34) pyelonephritis in the anamnesis 17.1% (OR = 2.22), non-termination of pregnancy 14.3% (OR = 2.17) in the anamnesis and a preeclampsia of 14.3% (OR = 2.16) in the anamnesis [1, 3, 4, 5, 8].
2. During the physiological course of pregnancy, as the duration of hestation increases, the level of PlGF in the

blood serum increase of level occurs: It was 108.3±8.24 pg/ml at 10-14 weeks of gestation and 222.2±22.64 pg/ml at 20-22 weeks of gestation. During pregnancy complicated by moderate preeclampsia, serum PlGF levels in the blood serum were 2.5 times lower than in the control group (42.6±1.33 pg/ml, $p<0.001$) during 10-14 weeks of gestation and its content was 61.7±1.52 pg/ml ($p<0.001$) during 20-22 weeks of gestation. In pregnant women with severe preeclampsia during 10-14 weeks of gestation, levels of PlGF were 13.4 times lower than in pregnant women in the control group (8.1±0.26 pg/ml, $p<0.001$) and 5.3 times lower than in pregnant women with moderate preeclampsia ($p<0.001$) and it was 11.3±0.52 pg/ml at 20-22 weeks of gestation [6, 9].

3. Measurement of PlGF levels in pregnant women with preeclampsia is of prognostic importance in the development of preeclampsia, especially in the development of its different severity, at different stages of gestation.Level of PlGF <45 pg/ml at 10-14 weeks of gestation is of greater diagnostic value in the development of severe preeclampsia (OR=3,28) and level of (OR=3,28) during 20-22 weeks of gestation [2, 6, 9, 10].
4. The development of preeclampsia and its severity is characterized by an increase in the level of inflammatory cytokines as the duration of hestasis increases. During moderate preeclampsia, TNF-α levels increased to 55.8±0.40 pg/ml during 10-14 weeks of gestation and to 74.5±0.68 pg/ml during severe preeclampsia ($p<0.001$). TNF-α levels during 20-22 weeks of gestation are 58.7±0.43 pg/ml in moderate preeclampsia and 75.0±1.11 pg/ml ($p<0.001$) in severe preeclampsia. The amount of IL-6 is 50.1±0.33 pg/ml in moderate preeclampsia during 10-14 weeks of gestation, and 62.3±0.80 pg/ml in severe preeclampsia ($p<0.001$). IL-6 levels during 20-22 weeks of gestation are 52.7±0.27 pg/ml in pregnant women with moderate preeclampsia and 63.9±0.43 pg/ml in severe

preeclampsia ($p<0.001$). Hyperplasia of inflammatory cytokines aggravates the course of preeclampsia in the later stages of pregnancy and is considered an unfavorable prognostic sign.Complex determination of PlGF levels with cytokines allows to identify pregnant women at high risk for developing preeclampsia. In pregnant women with severe preeclampsia, a mild inverse relationship between PlGF and TNF-α ($r = -0.325$, $p<0.01$) is found at 10-14 weeks of gestation [6, 9].

5. Based on the individually predicted algorithm for the risk of developing preeclampsia, it was shown that the clinical and anamnestic indicators and angiogenic factors of pathogenetic significance are:during severe preeclampsia at 10-14 weeks of gestation period BCI≥25 kg/m^2 and PlGF <45 pg/ml, pyelonephritis and PlGF <45 in the anamnesis pg/ml during moderate preeclampsia for 20-22 weeks of gestation [2, 7, 10].

PRACTICAL RECOMMENDATIONS

1. In addition to standard general clinical examinations for predicting the risk of developing preeclampsia in the pre-clinical stage in high-risk groups and diagnosing the disease, it is important to determine the level of PlGF - placental growth factor.
2. The development of severe preeclampsia can be predicted at 10-14 weeks of gestation when PlGF <45 pg/ml and BMI≥25 kg/m^2; When moderate preeclampsia is PlGF <45 pg/ml and if pyelonephritis is availablein the anamnesis, can be predicted at 20-22 weeks of gestation.
3. By looking at the angiogen / antiangiogen ratio PlGF / IL-10 as mediators of the development of preeclampsia in the early stages of gestation, the decrease in the cost of this ratio progresses with the increase in gestational age.

4. When assessing the individual prognosis of the development of preeclampsia in early pregnancy, it is necessary to use an algorithm developed based on multivariate analysis of clinical-anamnestic and angiogenic predictors of this complication.

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LIST OF ABBREVIATIONS

ALAT – alanine aminotransferase
ASAT – aspartate aminotransferase
BMI – body mass index
BP – blood pressure
CI – confidence interval
DAT – diastolic blood pressure
Hb – hemoglobin
IL-6 – interleukin-6
IL-10 – interleukin-10
LR – likelihood ratio
PlGF – Placental Growth Factor
OR – odds ratio
SAT – systolic blood pressure
TNF-a – tumor necrosis factor
Se – sensitivity
Sp – specificity

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