

ARTICLE

GENETIC POLYMORPHISM OF VASCULAR SYSTEM, DYSFUNCTION OF THE ENDOTHELIUM AND THE BLOOD COAGULATION SYSTEM AMONG WOMEN WITH GESTOSIS (PREECLAMPSIA)

Tatiana V. Pavlova*¹, Larisa I. Maltseva²¹Dept. of Obstetrics, Kazan Federal University, Medical Sanitary Unit of FSAEI HE, Kazan, RUSSIA²Dept. of Obstetrics and Gynecology, Kazan State Medical Academy, Kazan, RUSSIA

ABSTRACT



The article presents the results of a study to determine the genetic risk of pre-eclampsia in primiparous women. We have examined 165 primiparous women: 75 pregnant women with different degrees of severity of preeclampsia; 40 ones from the high-risk group, 50 - practically healthy primiparous women. The examination has shown that the determination of genetic defects, the level of folic acid, angiotensin-converting enzyme, renin, angiotensin, intravascular coagulation markers is prognostically significant in the diagnosis of severe preeclampsia, premature detachment of the normally located placenta, placental insufficiency, syndrome of retarded fetal development and congenital malformations of fetus in primiparous women.

INTRODUCTION

KEY WORDS

gestosis, preeclampsia, genetic polymorphism, endothelial dysfunction, folate deficiency, hyperhomocysteinemia

Gestosis (preeclampsia) is one of the topical problems of modern obstetrics and remains the most severe complication of pregnancy. The frequency of preeclampsia does not tend to decrease and fluctuates in Russia from 7% to 29% [1, 2], largely determining the indicators of maternal mortality and infant morbidity rate [3, 4]. According to recent studies, the genetic predisposition to the development of preeclampsia may be up to 50% of all risk factors [5, 6, 7]. The genetic factors with which preeclampsia may be associated include: metabolic genes (GSTM1, GSTT1, GSTP1, EPHX, CYP1A1); the main histocompatibility complex (HLA-G, DQA1, DQB1, DRB1); blood coagulation systems (FV, FII, MTHFR, FGB, ITGB3, F7); endocrine system (ESR1, ESR2, INHA); lipid metabolism (LPL, APOE, PPARG, ADRB3); cytokines and growth factors (TNFA, IGFI, IL1Q, IL1A, IL1B, IL1RN, CTLA4); endothelium (NOS3, EDNI, GNB3, VEGF); vascular system (ACE, AGT, REN, AGTR1, AGTR2, PAI 1). The products of the genes of the renin-angiotensin system are among the most important regulators of blood pressure and the homeostatic function of the kidney, ensuring the maintenance of vital processes in the body.

In recent years, the existence of variants of the gene of the angiotensin-converting enzyme (ACE) has been discovered. The ACE gene is mapped on chromosome 17q23. When cloning the ACE gene, it has been revealed that either Insertion (I) is comprehended or Deletion (D) Alu repeat, consisting of 287 base pairs is absent in Intion 16 [8, 9]. The level of ACE in serum in the patients who are homozygous for D alleles exceeded almost twice the level of the enzyme in homozygous for I alleles and has an average value in heterozygous - I/D genotype [9, 10]. The angiotensinogen gene (AGT) is located on the long arm of the 1st chromosome at the locus lq42-q43. The plasmatic level of angiotensinogen reflects its level of expression. The most studied are the M235T and T174M variants of the AGT gene. The association of the T-allele and the T/T genotype with high blood pressure is for the M235T variant (replacement of methionine by threonine) [9, 11]. Polymorphism of the gene for angiotensin converting enzyme (ACE) and angiotensinogen (AGT) is poorly related to the frequency and course of chronic arterial hypertension (CAH) without pregnancy, but may be a risk factor for the development of hypertension in pregnant women.

The main endothelial factor of relaxation is nitric oxide (NO). It is involved in maintaining the tone of the vascular wall, thrombo genesis, neurotransmission, immune system reaction, etc. [8,10]. Currently, three isoforms of NOS have been identified: NOS1 - neuronal (nNOS) or brain (bNOS); NOS2 - inducible (iNOS) or macrophage (mNOS); NOS3 - endothelial (eNOS) [12]. Each of them has features in the mechanisms of action, localization, in the biological significance for the organism. In the endothelium, thrombocytes, arterioles, mesangial cells, etc. eNOS is localized in large quantities [8]. The allelic variants of this gene lead to decrease of the level of expression of NO-synthetase and, as a result, diminution of the organism's resistance to hypertensive states from the external and internal environment.

From the genes of the blood coagulation system, the most extensively studied is the polymorphism of the gene of methylenetetrahydrofolate reductase MTHFR. Two variants of the gene have been described, the most significant of which is the mutation of C677T. The individuals being homozygous for this mutation have the thermo ability of MTHFR and the reduced enzyme activity to about 35% of the mean value. This mutation is accompanied by an increase of the level of homocysteine, which is a risk of pre-eclampsia in pregnant women. Over frequency of the 677T allele was observed not only with preeclampsia, but also with other complications of pregnancy (placental abruption, fetal growth retardation, antenatal death of fetus, fetal neural tube defects). Another important aspect of the MTHFR-C677T mutation is folate-deficiency anemia,

Received: 8 May 2018
Accepted: 19 July 2018
Published: 12 Oct 2018

*Corresponding Author
Email:
Maria.Pavlova@kpfu.ru

which further aggravates hypoxia, the course of disseminated intravascular coagulation and, thereby, contributes to the progression of microcirculatory disorders and the aggravation of preeclampsia [13, 14].

Obviously, preeclampsia has a number of genes of predisposition, so identifying candidate genes for the development of preeclampsia can help in defining a risk group and conducting effective prevention of this severe pathology. Especially unpredictable pre-eclampsia is in primiparous women and its relationship with genetic factors is extremely interesting.

In connection with this, we conducted a study which aimed to determine the polymorphism of a number of genes of the vascular system, endothelial dysfunction and the system of blood coagulation and their role in the development of preeclampsia in primiparous women.

MATERIALS AND METHODS

We have examined 165 primiparous women in the 27-38 weeks of their pregnancy at the age of $25,4 \pm 5,43$ years: 75 pregnant women with different severity of preeclampsia (50 - with moderate degree, 25 - severe), 40 women without a pre-eclampsia clinic, but from the group of high risk (with obesity - 10, with hypertension before pregnancy - 10, with kidney disease - 20) and 50 practically healthy pregnant women. Written informed consent was taken from all the participants and the study was approved by the Institute ethical committee, Commission of the Department of the Obstetrics.

All the patients have had, in addition to general clinical checkup with assessment of the hemostasiogram (platelet aggregation: spontaneous and induced, fibrinogen, APTT, INR, XIIa dependent fibrinolysis, AT III, FMSC, PDP, plasminogen, protein C and S), the blood tested by the method of PCR in real-time on detection of the polymorphism of the genes AGT-M235T (angiotensinogen), ACE I / D (angiotensin converting enzyme), MTHFR-C677T (metylenetetrahydrofolate reductase), E298D polymorphism by restriction analysis and 4a / 4b polymorphism in the NOS3 gene. The gene NOS3-E298D, 4a / 4b is responsible for the synthesis of the enzyme - endothelial NO synthase (eNOS), which participates in the synthesis of nitric oxide by endothelium and, consequently, in the regulation of vascular tone, blood flow and blood pressure. The studied genes were assessed with all variants of their mutations (AGT-T235T, ACE D / D, MTHFR-T677T, NOS3-D298D, 4a / 4a). The level of folic acid (FA), angiotensin-converting enzyme (ACE) and renin-angiotensin (RA) was determined by the method of immune chemiluminescent analysis (ICA).

The standard method χ^2 was used for statistical analysis. In the case of a small quantity or absence of any genotypes (alleles), the exact two-sided Fisher test recommended for such situations was used to verify the reliability of the differences obtained. The relative risk (odds ratio - OR) of disease development in a particular genotype was calculated according to the standard formula $OR = ad/bc$, where a and b are the number of patients with and without a mutant genotype, respectively, and c d are the number of women in the control group also having and not having a mutant genotype. OR is indicated with a 95% confidence interval. To calculate the comparison of the average values of digital data, as well as to verify the reliability of the results obtained, we used the methods of estimating the difference between parts, analysis of average trends (t - Student's test), correlation analysis. The level of $p < 0,05$ was taken for the reliability of the differences.

RESULTS

As shown by the studies, among healthy primiparous women, normal variants of the studied genes are determined in 98% of cases [Table 1]. In 2% of pregnant women, single mutant variants of the genes AGT-T235T, ACE D / D, NOS3-D298D, 4a / 4a were established, which were not realized in obstetric pathology. The measures of RA, ACE in this group were within the norm ($1,7 \pm 0,3$, $3,3 \pm 1,2$ and $38,89 \pm 25,34$, respectively). The level of FA was reduced by 15% in 16 pregnant women and averaged $8,45 \pm 4,51$ ng / ml [Table 2].

Practically all obese pregnant women are in the risk group, 8 out of 10 have a mutant version of MTHFR-T677T with a slight decrease in FA and an increase in ACE. One of them had, at the same time, a small malformation of the fetus (non-closure of palate - cleft palate). A mutant variant of MTHFR-T677T was also detected in 7 out of 10 women with hypertensive syndrome, one of them - in combination with a mutant variant of the ACE D / D gene and a slight increase in the level of CR. -A mutant variant of the ACE D/D gene and polymorphism of the NOS3-E298D, 4a/4b gene, where the alleles D and 4a raise the risk of preeclampsia, placental insufficiency, FGRS, with potentiating factors was disclosed in 10 women with pyelonephritis. Such factors could be a two-fold elevation of renin and angiotensin with a simultaneous decrease in FA by 1,8 times, which were found in these women. On average, a significant decrease in folic acid with increased renin, angiotensin and ACE was found in at-risk pregnant women with developing preeclampsia in comparison with healthy pregnant women [Table 2]. Attention was drawn to the absence of changes in haemocoagulative parameters.

During the assessment of pregnant women with moderately expressed pre-eclampsia, mutant genes of MTHFR-T677T in 38% of cases (19 women) and ACE D / D in 46% (23) were revealed, a combination of these mutant variants was found in two patients. The heterozygous variant of MTHFR-C677T was determined in a third of the primiparae of this group, which was clinically realized in placental insufficiency

and in the Syndrome of fetal growth retardation of I-II intensity in 7 pregnant women. Nine women with a mutant variant MTHFR-T677T had also FGRS of II intensity, long-lasting intrauterine hypoxia, oligohydramnios, at the same time, one of them had a partial detachment of the normally located placenta at 37 weeks pregnancy. The normal variant of the MTHFR-C677C gene was detected in 32% (16 pregnant women). The mutant gene for the D-NOS3-D298D, 4b / 4b allele was found in 30% (15 patients), all of them had clinical FGRS hypamnion. In the haemostasiogram of women with mild pre-eclampsia having mutant genes, an increase in D-dimer, fibrinogen, plasminogen, decrease in AT-III were revealed, i.e. manifestations of the syndrome of DIC.

Table 1: The results of the study the Allele frequencies of the polymorphic variants studied

Subjected groups	Evaluated polymorphism														
	MTHFR-C677T			ACE-I/D			AGT-M235T			eNOS-E298D			eNOS-4a/4b		
	CC	CT	TT	II	ID	DD	MM	MT	TT	EE	ED	DD	bb	ab	aa
Healthy pregnant women (n=50)	0,6*	0,33*	0	0,35*	0,54	0,11*	0,73*	0,27*	0	0,86*	0,24*	0	0,7	0,3*	0
Pregnant women at risk of preeclampsia (n=40) of them:	0,2*	0,18*	0,54*	0,6*	0,23*	0,17*	0,62*	0,38*	0	0,46	0,54*	0	0,6*	0,4*	0
With obesity (n=10)	0,1*	0,1*	0,8*	0,7	0,3*	0	0,6*	0,4	0	0,5*	0,5	0	0,7*	0,3*	0
With hypertensive syndrome (n=10)	0,2*	0,1*	0,7*	0,8*	0,2*	0	0,9	0,1*	0	0,4*	0,6*	0	0,6*	0,4	0
With renal diseases (n=20)	0,5*	0,45*	0	0,3*	0,2	0,5*	0,35*	0,65*	0	0,5	0,5*	0	0,5	0,5*	0
The group with moderate preeclampsia (n=50)	0,2*	0,34*	0,38*	0,3*	0,24*	0,46	0,34*	0,48	0,66*	0,5	0,2*	0,3*	0,44*	0,54*	0,02*
The group with severe preeclampsia (n=25)	0,08*	0,12*	0,8*	0,32*	0,24*	0,56	0,4*	0,32*	0,28	0,28*	0,4*	0,32*	0,12*	0,24*	0,64*

Note: n – the number of individuals in a group, *- accuracy of allele frequencies by comparing the subjected groups with a control group (p<0,05)

Table 2: The level of Folic Acid, Angiotensin-Converting Enzyme, Renin-Angiotensin in the studied groups

Subjected groups	FA, ng/ml	ACE, U/l	Renin, ng/ml/h	Angiotensin, ng/ml
Healthy pregnant women (n=50)	8,45±4,51*	38,89±25,34*	1,7±0,3*	3,3±1,2*
Pregnant women at risk of preeclampsia (n=40)	6,15±2,51*	55,46±18,14	2,54±1,27*	5,01±2,75*
Pregnant women with moderate preeclampsia (n=50)	3,08±1,64*	71,01±21,04*	4,54±2,27*	8,03±2,79*
Pregnant women with severe preeclampsia (n=25)	2,37±1,12**	89,99±24,01*	6,21±2,8*	10,64±3,66*

Note: n – the number of individuals in a group, *- accuracy of allele frequencies by comparing the subjected groups with a control group (p<0,05)

The majority of pregnant women with severe preeclampsia – 73,2% (18) had a combination of mutant gene variants: 56% of them had ACE D/D and MTHFR-T677T (14). In 9 of them, a mutant variant of the AGT-T235T gene was also disclosed [Table 1]. In one woman, mutation of MTHFR-T677T was clinically realized in premature detachment of the normally situated placenta at pregnancy of 27 weeks. In this group, there was also a significant increase in ACE (89,99 ± 24,01), a triple increase in D-dimer values, hyperfibrinogenemia, expressed thrombocyte hyper-aggregation, decrease in AT-III, which corresponded to the active DIC syndrome.

Women with severe preeclampsia in 65% [16] had a mutant variant of the gene for allele 4a – NOS3-4a/4a. This was accompanied by early edema, proteinuria, as well as an active course of DIC syndrome. In 36% [9], the homozygous form of D298D polymorphism of the NOS3 gene was established [Table 1]. In this case, the FGRS of II - III with the progressive increase in the severity of preeclampsia was clinically defined.

Statistical analysis confirmed that NOS3-D298D and NOS3 4a/4a genotypes are significantly more frequent in women with preeclampsia than in patients at risk ($\chi^2 = 5.81$, $p < 0,05$ and $\chi^2 = 6,03$, $p < 0,05$, respectively).

The results of our studies have shown that the presence of the mutant variant of the gene for the allele 4a – NOS3-4a/4a is always accompanied by earlier edema, proteinuria and, as a rule, the formation of FGRS of severity II-III. The highest level of basal NO corresponds to genotype 4b/4b, whereas the level of NO was 2 times lower in women with genotype 4a / 4a. Heterozygous mutation forms occupy an intermediate position in terms of NO. Association 4a/4a of the genotype of the NOS3 gene with the development of preeclampsia made it possible to estimate the relative risk of this complication in pregnant women as significant – 1,74 (CI-95%: 1,06-2,97).

A comparative analysis of the polymorphism of the ACE I/D gene has shown that the DD genotype in women with preeclampsia is found 6,1 times more often (48,8%) than in healthy women (8%). In severe cases, the genotype of DD is found in 36% of pregnant women ($\chi^2 = 4,82$, $p < 0,05$). In the ACE I/D polymorphism, the presence of D allele is associated with the development of preeclampsia (OR 2,15 and CI 95%: 0,77-5,27) and should be considered as a risk factor of the development of preeclampsia, whereas in the analysis of frequencies of genotypes and alleles of AGT -M235T in all groups, significant differences are not revealed.

According to the indication of genetic diversity - PIC (informational content of polymorphism), which gives an idea of how informative the selected marker is, all the studied genetic parameters (I/D, M235T, E298D, 4a/4b, C677T) turned out to be moderately informative (0,319; 0,331; 0,366; 0, 316, respectively), which proves their role in the development of pre-eclampsia in primiparous women.

DISCUSSION

The reasons for the development of preeclampsia in women during pregnancy remain unclear and much attention to the development of the genetic mechanisms of this complication of pregnancy is understandable.

It is quite obvious that pre-eclampsia is a polygenic pathology. A lot of data on the expression of various genes, encoding ACE, angiotensin, endothelin, synthycin 1 and 2, involved in stimulating the cytotrophoblast cells migration and placenta development, endothelial vascular growth factor, methylenetetrahydrofolate reductase, etc. There are data on changes in HLA antigen alleles as the cause of pre-eclampsia development [3, 10]. Of particular interest are primiparous women who do not have a severe obstetrical anamnesis, and that is why they are related to an unpredictable category of patients, as well as at-risk women - with obesity, hypertension, renal diseases.

The results of our studies have shown that both primiparous women with preeclampsia and a group of high-risk development have common genetic changes. A mutant variant of the MTHFR-T677T gene was found in pregnant women with obesity and hypertensive syndrome (80%). The patients with pyelonephritis had the mutant variant ACE D/D dominated, which gives the most severe course of preeclampsia. The rest had heterozygous variants of AGT-M235T, NOS3-E298D, 4a/4b and single variants of their mutations.

Preeclampsia of moderate severity was accompanied by a mutant MTHFR-T677T gene in 38% of cases and ACE D/D in 46%, a heterozygous variant was found in every third pregnant woman, in combination with placental insufficiency and FGRS. The mutant variant of the gene of the D –NOS3 D298D allele leads to similar complications.

Most women with severe preeclampsia have a combination of mutant ACE D/D genes, MTHFR-T677T, and a mutant variant of the AGT-T235T gene is revealed in 50%. In addition, in severe form, as a rule, a mutant variant of the gene of the allele 4a–NOS3 4a/4a with an early onset and progressive increase in severity is identified. It should be noted that the changes in the genetic portrait of women with preeclampsia were probably defined by other equally severe obstetric complications - FGRS in 46%, chronic fetoplacental insufficiency with fetal hypoxia in 33%. Also, the mutation in the MTHFR gene in two cases was clinically accompanied by a partial detachment of the normally situated placenta at pregnancy of 27 and 37 weeks.

It is important to emphasize that the mutation of the studied genes in pregnant women with preeclampsia has always been combined with an abrupt decrease in the level of FA (by 60-80% of the norm) and a significant increase in RA [Table 2].

As it turned out, the decrease in values of FA with the normal variant of the MTHFR-C677C gene during pregnancy does not lead to the development of hypertensive syndrome, but is realized by a greater frequency of placental insufficiency and FGRS.

A low level of FA with an increase in RA against the background of the expression of mutant genes is accompanied by a disruption in the system of hemostasis and hypertensive syndrome of varying severity. The initial signs of DIC syndrome were observed with moderate severity of preeclampsia, progressive DIC syndrome - with severe course.

However, assessment of changes in the parameters of folic acid, ACE, renin and angiotensin requires special attention. A significant correlation between the level of FA, ACE, renin, angiotensin and the severity of preeclampsia ($r = -0,65$; $r = 0,57$; $r = 0,72$; $r = 0,68$, respectively; $p < 0,05$) was found.

The data obtained have shown that the decrease in FA with increasing ACE, renin, angiotensin is observed in women at risk and with the increase in the severity of preeclampsia, the values of FA progressively decrease by 60-80% of the norm, while the values of ACE, renin, and angiotensin increase 1,6; 2,5 and 2 times, respectively, compared with women at risk [Table 2].

Probably the mutant MTHFR genes found in women at risk and in those ones with preeclampsia lead to an imbalance of folate metabolism and even the prescription of synthetic folic acid is unable to change the situation with folate deficiency and the formation of endotheliopathy. The mutant genes of renin, angiotensin, nitrous oxide increase endothelial dysfunction with the development of vascular complications and severe obstetric syndromes.

Thus, the detection of genetic defects, the levels of folic acid, ACE, renin, angiotensin, and intravascular coagulation markers can be prognostically significant in the diagnosis of severe pre-eclampsia, premature detachment of the normally located placenta, placental insufficiency, FGRS and fetal malformations in primiparous women, who do not have a burdened obstetrical anamnesis.

CONCLUSION

The development of pre-eclampsia in primiparous women is genetically determined. Polymorphism of the genes of nitrogen oxide – NOS3-E298D, 4a/4b, mutation in gene of angiotensin converting enzyme – ACE D/D with pyelonephritis, mutation in gene of methylene tetra hydro folate reductase – MTHFR-T677T with chronic hypertensive states and obesity form a risk group of women with a high potential for realization in preeclampsia. The development of mild pre-eclampsia is determined by the mutation of one of the genes: MTHFR-T677T, ACE D/D, or NOS3-D298D, 4a/4a, combined with a 60% decrease in folate levels and a 2-fold elevation of the ACE in blood in pregnant women. A severe form of pre-eclampsia occurs with a combined mutation of MTHFR-T677T and ACE D/D or the presence of the NOS3-D298D, 4a/4a genes with reduced folate content by 80% compared to healthy primiparae and an increase in ACE by more than 2 times.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

FINANCIAL DISCLOSURE

None

REFERENCES

- [1] Radzinsky VE. [2004] Immunologic and Genetic Predictors of Gestosis and FGRS. Materials of the 36-th Annual Congress of International Society on Pathophysiology of Pregnancy of Organization of Gestosis. 187-188.
- [2] Radzinsky VE, Orazmuradov AA, Knyazev SA, et al. [2006] Reduction in Obstetric Aggression with Pregnancy of Low perinatal Risk Herald of Russian University of Peoples' Friendship, series Medicine. 4:5-12.
- [3] Ailamazyan EK, Mozgovaya EV. [2008] Gestosis Theory and Practice. M Medpress Inform. 271.
- [4] Radzinsky VE, Kuznetsova OA, Aleyev IA, et al. [2006] Controversies in Obstetrics (Obstetrics Roundup. «The 7th world congress on controversies in obstetrics, gynecology and infertility». Athens. Greece, 2005) Obstetrics and Gynaecology. 2:59-62.
- [5] Ginter EK. [2006] Medical Genetics Textbook. M Medicine. 131-141.
- [6] Mozgovaya EV. [2003] Estimate of Genetic Predisposition to Gestosis: Polymorphism of Genes in Regulation of the Function of Endothelium. Journal of Obstetrics and Diseases of Women. 3(2): 25-34.
- [7] Ness RB, Roberts JM. [2006] Heterogeneous causes constituting the single syndrom of preeclampsia: A hypothesis and its implications. Am. J. Obstet. Gynecol. 175:1365-1370.
- [8] Strizhakov AN, Bayev OR, Ignatko IV. [2006] Prediction of Gestosis Development and Feto placental Insufficiency. Russian Journal of Obstetrician Gynecologist. 1:39-42.
- [9] Arngrimsson R, Hayward C, Nadaud S. [1997] Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region Am J Hum Genet. 61:354-362.
- [10] Cooper DW. [2007] Genetics of Hypertension in pregnancy: possible single gene control of preeclampsia and eclampsia. Am J Obstet Gynecol. 53: 851-863.
- [11] Ookif Takakuwa K, Akashi M, et al. [2008] Am J Reprod. Immunol. 60(1):75 – 84.
- [12] Frost P, Blom H, Milos R, et al. [2005] A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase Nat. Genet. 10:111-113.
- [13] Zhou N, Chen J, et al. [2004] Detection of insertion deletion polymorphism of angiotensin converting enzyme gene in preeclampsia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 16(1):29-31.
- [14] Williams MA, Sanchez SE, et al. [2004] Methylenetetrahydrofolate reductase 677 C T polymorphism and plasma folate in relation to preeclampsia risk among Peruvian women. Matern. Fetal Neonatal Med. 15:337-344.