ORIGINAL ARTICLE

RISK OF PLACENTA-ASSOCIATED COMPLICATIONS AT PREECLAMPSIA IN PREGNANT WOMEN WITH THROMBOPHILIA

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ABSTRACT

The aim: To study the distribution and influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in women with preeclampsia (PE).

Materials and methods: The prospective cohort study included 46 women with PE and maternal or fetal complications and 87 pregnant women with PE, without complications. Genetic polymorphisms of coagulation factors and fibrinolysis (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), endothelial dysfunction (192 Q→R PON-1, 677 C→T MTHFR) and blood pressure regulator (235 M→T angiotensinogen II) were studied with the help of allele-specific polymerase chain reaction

Results: Markers of predisposition to the development of obstetric and perinatal complications in pregnant women with PE are the following genotypes: 1691 GA by V Leiden factor gene – increases the risk in 2.9 times (95% CI 1.94-4.33), 20210 GA by prothrombin gene – in 2.36 times (95% CI 1.54-3.6), 20210 AA by prothrombin gene – in 3.12 times (95% CI 2.4-4.0). Pathological polymorphisms in the genes of angiotensinogen II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, paraoxonase-1 192 Q→R do not significantly affect the development of complications during preeclampsia.

Conclusions: The development of PE against the background of the existence of acquired and hereditary types of thrombophilia is associated with a more severe course, early-onset and the development of life-threatening complications for a mother and fetus.

KEY WORDS: MTHFR, factor V Leiden, prothrombin, abruptio placentae, fetal growth retardation, plasminogen activator inhibitor 1

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INTRODUCTION

Preeclampsia (PE) occurs in 5-8% of pregnancies and is the leading cause of maternal and perinatal morbidity and mortality [1-3]. The main threat is preeclampsia-related complications during and after pregnancy. Complications include: abruptio placentae (AP), premature birth, bleeding, eclampsia, fetal growth retardation (FGR), fetal distress and intrauterine fetal death (IFD). Non-obstetric complications include heart failure, pulmonary edema, cardiomyopathy, cerebrovascular disorders, retinal detachment, and an increased risk of cardiovascular disease in future [3,4].

Basic studies have shown that PE is associated with systemic inflammatory response, endothelial dysfunction, oxidative stress, imbalance of angiogenic and antiangiogenic factors, metabolic disorders [5,6]. The starting point of these pathological processes is considered to be inadequate trophoblast invasion [6]. It is now clear that PE, along with premature birth, fetal growth retardation, intrauterine fetal death and other conditions of pregnancy is a multifactorial disease. All of the above conditions are called "major obstetric syndromes" [7,8] that are associated with insufficiently deep placentation that may be associated with varying degrees of reduced remodeling and obstructive damage to the spiral arteries in the joint area or in the myometrium. The introduction of the term "major obstetric syndromes" was intended to explain the failure of works on the prediction and prevention of obstetric diseases, drawing the attention of researchers and clinicians to the etiological heterogeneity of conditions that share common pathogenetic pathways [7]. Common for conditions combined into "major obstetric syndromes" are: etiological heterogeneity, long preclinical phase, fetal disease, ineffective symptomatic treatment, clinical implications have adaptive characteristics, genetic factors and environmental factors play a role in the occurrence.

In recent years, significant progress has been made in the screening and prevention of PE [9,10], but the expected success in the treatment of PE has not been achieved. This is probably due to the fact that PE is the ultimate clinical implication of disorders of different origins. Indeed, the nature of the disease is different if it has developed before 34 weeks or at almost full term pregnancy. Not every case of PE is accompanied by complications, i.e. there is also noted relatively favourable course of the disease. Recently, there has been emerged the evidence of two types of PE: early and late. Early preeclampsia (EOPE) is accompanied by placental dysfunction, increased markers of endothelial dysfunction, early onset, severe course, development of complications and more often ends in induced premature

birth [1]. Maternal mortality is 12 times higher with the development of PE up to 28 weeks of pregnancy, and the period of 34 weeks is most often considered critical [11,12], as it correlates with abnormal placentation in the early stages. Late one (LOPE) is mostly associated with "maternal contribution": metabolic syndrome, hypertension, rarely accompanied by FGR and has a relatively favourable course [7].

The "combination" of EOPE and LOPE in study and development of clinical strategies may complicate the further study of the pathophysiology of this syndrome and the achievement of significant clinical results. Studies on the association of preeclampsia with thrombophilia are conflicting. Clinical heterogeneity of the disease may be one of the explanations [11-13]. A preliminary hypothesis in our study is that the development of maternal and fetal complications in PE or their absence are determined by various pathogenetic mechanisms, namely the existence of pathological genes of polymorphisms (hereditary thrombophilia, mutations in genes of blood pressure regulators and endothelial dysfunction) that are additional factors that cause, maintain and enhance reduced placental perfusion.

THE AIM

To study the distribution and influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in women with PE.

MATERIALS AND METHODS

The study was conducted at Dnipro State Medical University, Dnipro, Ukraine, in 2018-2020. A prospective cohort study covered 133 women in the second half of pregnancy. The criterion for inclusion into the study is the presence of PE in accordance with the recommendations of the ISSHP [14].

The main cohort (M) consisted of 46 women with PE and maternal or fetal complications. As complications there were considered premature detachment of the normally located placenta (4 women – 8.7%), eclampsia (1 – 2.17%), HELLP syndrome (1 – 2.17%), FGR (23 – 50.0%), IFD (6 – 13.04%), fetal distress during pregnancy (21 patients – 45.65%). The comparator group (C) was formed by 87 pregnant women with PE, without complications. The study did not include pregnant women with a physiological course of pregnancy, since the purpose of the study was to determine the influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in preeclampsia.

Genetic polymorphisms of coagulation factors and fibrinolysis (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), endothelial dysfunction (192 Q→R PON-1, 677 C→T MTHFR), blood pressure regulator (235 M→T angiotensinogen II) were studied with the help of allele-specific polymerase chain reaction, followed by detection by electrophoresis in 3% agarose gel. A set of reagents "SNP-Express" (Litech SPF, Russian Federation) was used. DNA from leukocytes of blood, which was isolated using the reagent "DNA-express blood" (Litech SPF, Russian Federation) was used for analysis.

Statistical processing of the study results was performed using licensed computer programs Microsoft Excel 2010 and Graph Pad Prism 5 using methods of parametric and nonparametric statistics. The normality of the distribution of quantitative traits was assessed using Shapiro-Wilk and Kolmogorov-Smirnov criteria, analysis of variance, odd t-test, Mann-Whitney test, $\chi 2$ test with conjugation of conjugation tables and Yates correction, Fisher's exact test were used. Spearman and Pearson correlation coefficients (r) were used to assess the relationship between the indicators. To assess the relationship between impact and outcome, relative risk (RR) and odds ratio (OR) assessments were performed at 95% confidence interval (CI).The difference between the values was considered significant by $p<0.05$ [2,8].

The management of the study was conducted in full compliance with the ethical principles contained in the "Human Rights Declaration" adopted in Helsinki, which follows the Good Practice Rules in the Clinical Study and Legal Regulations and with the approval of the Ethics Committee of the Dnipro State Medical University.

RESULTS

The average age of women and the distribution by age categories between groups almost did not differ: in M group – 26.98 ± 0.9 years, in C group – 28.98 ± 0.65 ($p>0.05$).

Analysis of reproductive function showed that the number of women with a history of childbirth and the average number of births per woman in the group with obstetric complications was less than in the group without complications. Accordingly, the number of first-borns in the M group was significantly higher 35 (76.09%) than in the comparator group 44 (50.57%) ($p <0.05$, OR = 3.1; 95% CI 1.4-6.9, RR = 1.5, 95% CI 1.16-1.96). PE equally often complicated obstetric history in the study groups.

The mean period of PE onset in M group (29.02 ± 0.55 weeks) was 1.19 times shorter than in C group (34.45 ± 0.25 weeks) ($p<0.001$). The duration of PE in the group with complications (4.48 ± 0.47 weeks) is 1.7 times longer than in C group (2.63 ± 0.18 weeks, $p<0.001$). It was found that the onset of preeclampsia at 28 weeks of gestation or earlier increases the relative risk of complications by 34.04 times ($p<0.001$, RR = 34.04, 95% CI 4.69-247.1), and the duration of PE for more than 5 weeks increases the relative risk of complications by 3.57 times (95% CI 1.73-7.38).

The mean gestational age at the time of delivery in M group (33.50 ± 0.57) was 3.44 weeks less than in the comparator group (36.94 ± 0.27, $p<0.001$). This is due to the severity of preeclampsia and the development of complications that required preterm birth. In the main cohort, 7 (15.22%) women had mild preeclampsia; this is

Table I. Frequency of genotypes and alleles in pregnant women from study groups, n (%)

Study group	Genotype		
ANG 235 M→T	MM	MT	TT
M (n=46)	10 (21.74)	22 (47.83)	14 (30.43)
C (n=87)	31 (35.63)	34 (39.08)	22 (25.29)
prothrombin 20210 G→A	GG	GA	AA
M (n=46)	31 (67.39)*	10 (21.74)*	5 (10.87)*
C (n=87)	83 (95.4)	4 (4.6)	0 (0.0)
FVL 1691 G→A	GG	GA	AA
M (n=46)	26 (56.52)*	19 (41.3)*	1 (2.17)
C (n=87)	80 (91.95)	7 (8.05)	0 (0.0)
PAI-1 5G/4G	5G/5G	5G/4G	4G/4G
M (n=46)	7 (15.22)	30 (65.22)	9 (19.57)
C (n=87)	22 (25.29)	44 (50.57)	21 (24.14)
fibrinogen β 455 G→A	GG	GA	AA
M (n=46)	16 (34.78)	26 (56.52)	4 (8.7)
C (n=87)	39 (44.83)	37 (42.53)	11 (12.64)
MTHFR 677 C→T	CC	CT	TT
M (n=46)	21 (45.65)*	17 (36.96)	8 (17.39)
C (n=87)	60 (68.97)	19 (21.84)	8 (9.2)
PON-1 192 Q→R	QQ	QR	RR
M (n=46)	28 (60.87)	13 (28.26)	5 (10.87)
C (n=87)	41 (47.13)	31 (35.63)	15 (17.24)

Note: * – the statistical significance of differences of indicator relative to the P group ($p<0.05$), the $\chi 2$ test and Fisher's exact test are used.

less than in the comparator group (57 – 65.52%, $p <0.001$; OR = 0.09; 95% CI 0.04–0.25). In the group with complications, severe preeclampsia had 39 (84.78%) women, which is more than in C group – 30 (34.48%) ($p <0.001$; OR = 10.59; 95% CI 4.23- 26.5, RR = 2.46, 95% CI 1.79–3.37). Edema of varying severity was noted in most women of both groups: 41 (89.13%) persons of M group, 69 (79.31%) of C group ($p> 0.05$).

Analysis of the results of tests of genes that regulate the hemostasis system, "endothelial system" and genes that regulate blood pressure, revealed a high frequency of pathological polymorphisms in patients with preeclampsia, regardless of complications: 46 (100%) women of M group and 84 (96.5) %) of C group (Table I). The results of testing for the presence of polymorphisms in the gene ANG II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, PON-1 192 Q→R did not reveal significant changes between the studied groups (Table I).

DISCUSSION

Considering the comparative analysis of frequencies of genotypes and alleles of the FVL 1691 G → A gene (Table I), the frequency of normal homozygotes GG was reduced 1.62 times in the M group compared to the C group ($p <0.001$, OR = 0, 11, 95% CI 0.04-0.3, RR = 0.33, 95% CI 0.22-0.49), and an increase in the number of heterozygotes GA in the M group 5.13 times compared with the C group <0.001, OR = 8.04, 95% CI 3.05-21.22). In carriers of the GA FVL genotype the risk of complications during PE is increased by 2.9 times (95% CI 1.94-4.33). High prevalence of Factor V Leiden 1691G/A variation in preeclamptic patients was determined in a study of Ahmed, N. A. et al [15].

Analyzing the frequency of prothrombin gene genotypes (20210 G→A), it is found that mutations are unique to the group with preeclampsia and complications. In M group, the frequency of heterozygous forms exceeded 4.73 times the rate of C group ($p <0.05$, OR = 5.7; 95% CI 1.7-19.6; RR = 2.36, 95% CI 1, 54-3.6). The frequency of normal homozygotes of GG in M group is 1.42 times less than in C group ($p <0.001$, OR = 0.1, 95% CI 0.03-0.32; RR = 0.34, 95% CI 0, 24-0.5). The number of mutant homozygotes 20210 AA of the prothrombin gene is significantly higher in the M group than in C group ($p <0.05$, OR = 23.19, 95% CI 1.25-429.4), and the risk of complications during PE in carriers of this genotype is increased by 3.12 times (95% CI 2.4-4.0). Our data confirm previous studies [12,15,16].

Analysis of the frequencies of alleles and genotypes MTHFR 677 C→T revealed a decrease in the frequency of the normal genotype of CC in the M group. Its frequency is reduced by 1.5 times compared with C group ($p <0.05$, OR = 0.38, 95% CI 0.18-0.79; RR = 0.54, 95% CI 0.34-0.

86). The number of heterozygotes 677 CT MTHFR, pathological homozygotes 677 TT between study groups did not differ significantly. Thus, pregnant women with *wild-type CC MTHFR 677* had a protection effect against PE complications, that is consistent with Yang, Y. L. et al [17].

Allelic polymorphisms of ANG II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, paraoxonase-1 192 Q→R were equally common regardless of the presence of complications. That is, the presence of the most pathogenic polymorphisms and their combined action play a role in the occurrence of complications in PE. This is confirmed by correlations between mutations in the prothrombin genes 20210 G→A, FVL, MTHFR 677 C→T and the development of obstetric and perinatal complications in PE r = 0.387, r = 0.421 and r = 0.225, respectively ($p < 0.05$). In a study [16] significant differences in MTHFR A1298C, C677T and FVL polymorphisms between EOPE and LOPE were proved. The synergic effect of MTHFR variants could increase PE and EOPE risk.

It has been suggested that the presence of several pathological genes or polymorphisms is important for the occurrence of complications in pregnant women with preeclampsia, and their pathological effect is summed up. Three or more combined polymorphisms were mostly (2.78 times) determined in the M group (54.3%) than in the C group without complications - 19.5% ($p < 0.001$, OR = 2.58, 95 % CI 1.64-4.05). The adverse effect of pathological polymorphisms on the development of perinatal complications is explained by disorders in the hemostasis system: increased aggregation, adhesion of platelets, coagulation properties of blood, which leads to impaired microcirculation in the utero-placental system.

It has been found that the development of complications in PE is accompanied by ($p < 0.05$): early (1.19 times) onset of the disease (29.02 ± 0.55 vs. 34.45 ± 0.25 weeks), increased (1.7 times) duration of hypertension (4.48 ± 0.47 weeks vs. 2.63 ± 0.18 weeks), less (3.44 weeks) gestational age at the time of delivery (33.50 ± 0.57 vs. 36, 94 ± 0.27 weeks), lower weight of newborns – 1.79 times (1647 ± 103.2 g vs. 2951 ± 71.68 g) and newborns body length – 1.18 times (42.5 ± 0.85 cm vs. 50, 25 ± 0.41 cm), lower Apgar scale score at 1st minute – 1.28 times (5.1 ± 0.32 vs. 5.53 ± 0.24 points), and at 5th minute – 1.25 times (6.07 ± 0.36 against 7.64 ± 0.06 points). Previous report by Lisonkiva S et al [18], showed that early- but not late-onset preeclampsia conferred a high risk of perinatal death/severe neonatal morbidity 16.4 (95% CI, 14.5-18.6) in early-onset and 2.0 (95% CI, 1.8-2.3) in late-onset preeclampsia.

Summarising the above, it should be assumed that there are two types of hypertensive disorders during pregnancy: the first is a severe PE that begins early and is accompanied by life-threatening complications for mother and child, and the second is a mild PE, in late pregnancy and without concomitant complications. Various etiological and pathophysiological changes lie in the development of these types of hypertensive disorders, which differ in time and course. Our opinion is consistent with Lisonkova S. [18], Simcox L. E. et al.[13].

CONCLUSIONS

The development of PE against the background of hereditary types of thrombophilia, namely polymorphism 1691 GA by factor V Leiden, 20210 GA by prothrombin gene, multigenic forms of thrombophilia is associated with more severe course, early onset and development of life-threatening complications for mother, fetus and newborn.

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