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The Level of Fibrinogen and Gene Polymorphism in Pregnant Women with Placental Insufficiency

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Abstract
Objectives: To study the association of genetic polymorphisms of coagulation factors with clinical and laboratory indicators in pregnant women with placental insufficiency with fetal growth retardation. *Materials and methods:* The study group included 250 pregnant women with placental insufficiency with fetal growth retardation (FGR). The control group consisted of 247 women without FGR. The participants were genotyped for four genetic markers of hereditary thrombophilia: fibrinogen (-455G/A *FI*), prothrombin (20210G/A *FII*), factor V Leiden (1691G/A *FV*), proconvertin (10976G/A *FVII*). *Results:* In the group of pregnant women with FGR, having genotypes -455GA *FI* and -455AA *FI*, a higher level of fibrinogen (4.7 g/l) is observed compared to women with the genotype -455GG *FI* (4.5 g/l, p=0.04). *Conclusion:* Thus, the study conducted on the relationship of polymorphic locus -455G/A *FI* with a higher level of fibrinogen in pregnant women with fetal growth retardation.

Keywords: *Placental Insufficiency, Fetal Growth Retardation, Single Nucleotide Polymorphism, Pregnancy.*

Introduction

Placental insufficiency is a syndrome caused by morphofunctional changes in the placenta, the progression of which develops a fetal growth retardation syndrome, often combined with hypoxia (Bamfo, Odibo, 2011, Zollner et al., 2011; Unanyan et al., 2015). Placental insufficiency (PI) is one of the most common complications of pregnancy (Serov et al., 2011; Diner et al., 2016). Perinatal mortality in women who underwent placental insufficiency is among the full-term newborns 10.3 %, among preterm infants – 49 % (Serov et al., 2011; Diner et al., 2016). In 60% of cases, placental insufficiency leads to the development of FGR, which ranks third in the structure of the causes of perinatal morbidity (Serov et al., 2011). Placental insufficiency has a multifactorial nature (Makacariya, Bicadze, 2006, Serov et al., 2011; Zollner et al., 2011).

One of the factors leading to the development of placental insufficiency and FGR is hereditary thrombophilia (Zotz et al., 2008; Facco et al., 2009; Nishizawa et al., 2011; Kosar et al., 2011).

But the results of these studies, obtained by different authors, are often contradictory (Camilleri et al, 2004; Infante-Rivard et al., 2005; Dudding et al., 2008; Zotz et al., 2008; Facco et al., 2009; Ivanov et al., 2009; Shanker et al., 2009; Kosar et al., 2011; Nishizawa et al., 2011; Coriu et al., 2014; Livrinova et al., 2015; Reshetnikov et al., 2017).

Materials and Methods

Object of Study

In total 497 unrelated pregnant women in the third trimester of pregnancy were recruited for the study during 2009-2013. All participants signed an informed consent before entering the study. The clinical and laboratory examination of the participants was conducted in the perinatal center of the Saint Joasaph Belgorod Regional Clinical Hospital. The following inclusion criteria were used to check eligibility of the participants: singleton pregnancy, Russian ethnicity. Patients having congenital malformations of internal genitals, uterine fibroids, anomalies of placental location, isosensitization of Rh factor or ABO, genetic diseases, were excluded from the study.

250 participants were diagnosed with the syndrome of fetal growth retardation of varying severity. The diagnosis of the syndrome was based on clinical data, ultrasound fetometry (TOSHIBA XARIO SSA-660A) and parameters of growth and weight after the birth. Based on the differences between the fetometric data and nomograms, three degrees of FGR were classified: 1st degree corresponded to the reduction of the estimated gestational ages from the standard ones by 2 weeks, 2nd degree – by 3-4 weeks, and 3rd degree – by more than 4 weeks.

The controls were 247 females without the syndrome of intrauterine growth retardation.

The laboratory study included a clinical blood test (with determination of the number of basic blood elements, hematocrit indicator); biochemical blood test (total protein, total bilirubin, urea, creatinine, level of hepatic aminotransferases, alkaline phosphatase, glucose); coagulogram (prothrombin index, activated partial thromboplastin time, thrombin time (TB), fibrinogen, international normalized ratio (INR), ethanol test, b-naphtholol test), extended study of

hemostatic system (detection of soluble fibrin monomer complexes), antithrombin-III, protein C and protein S, platelet aggregation tests); determination of blood type and Rh factor. Also, analyzes were performed to exclude the antiphospholipid syndrome (lupus anticoagulant, cardiolipin antibodies, antiphospholipid antibodies) and associated hereditary thrombophilia.

Molecular and Genetic methods

The participants were genotyped for four genetic markers of hereditary thrombophilia: fibrinogen (-455G/A *FI*), prothrombin (20210G/A *FII*), factor V Leiden (1691G/A *FV*), proconvertin (10976G/A *FVII*).

The material for the study was venous blood, obtained in a volume of 8-9 ml from the ulnar vein of pregnant women. All polymorphic variants of hereditary thrombophilia were analyzed using the method of polymerase chain reaction (PCR) of DNA synthesis in real-time (real-time-PCR).

Statistical Methods

The obtained genotypes were checked for their correspondence to the Hardy-Weinberg distribution (HWE) using the χ^2-test. Association of the polymorphisms with FGR was estimated by analyzing 2×2 contingency tables with the χ^2-test and the Yates' correction for continuity. The risk was estimated using odds ratio with 95% confidence intervals (95% CI). The median (Me) and interquartile range (Q25-Q75) were used to describe the traits studied, and the Mann-Whitney test for comparative analysis. The analyses were performed using STATISTICA 6.0 (StatSoft, USA). The Bonferroni correction was applied to adjust for multiple comparisons.

Results

Characteristics of pregnant women with FGR and control group are shown in Table 1.

The analysis of clinical and laboratory parameters in pregnant women with FGR and control group was carried out: a general blood test, a biochemical blood test, a coagulogram, as well as the level of antithrombin III and soluble fibrin-monomer complexes (SFMC), a general urine test. The associations of genetic variants of coagulation factors with clinico-laboratory indicators in pregnant women with FGR and in the control group were studied.

Table 1 Physical Characteristics and Various Medical Pathologies in the Study Participants

	FRG patients, n (%)	Controls, n (%)	p
N	250	247	
Age, y (min-max)	26.78 ± 4.81 (16.0-45.0)	26.20 ± 5.01 (19.0-41.0)	>0.05
BMI, kg/m^2	23.37 ± 4.31	23.90 ± 3.96	>0.05
Somatic Pathologies			
Essential Hypertension	50 (20.00)	29 (11.74)	0.02
Chronic Pyelonephritis	63 (25.20)	55 (22.27)	0.51
Obesity	23 (9.20)	22 (8.91)	1.00
Varicose Veins	13 (5.20)	10 (4.05)	0.69
Venous Thromboembolism at Pregnancy	4 (1.60)	1 (0.41)	0.38
Chronic Gastroduodenitis	26 (10.40)	22 (8.89)	0.68
Cerebrovascular Disease in History	2 (0.80)	0 (0.0)	0.50
Gynecological Pathologies			
Medical Abortion in History	80 (32.00)	65 (26.32)	0.20
Infertility in History	5 (2.00)	8 (3.24)	0.56
Miscarriage in History (Total)	46 (18.40)	25 (10.12)	0.01
Miscarriage in First Trimester	36 (14.40)	20 (8.10)	0.04
Pregnancy Loss in First Trimester	12 (4.80)	12 (4.86)	1.00
Ectopic Pregnancy	10 (4.00)	15 (6.07)	0.39
Disorders of the Menstrual Cycle in History	20 (8.00)	14 (5.67)	0.39
Pelvic Inflammatory Disease in History	58 (23.20)	59 (23.89)	0.94
Intrauterine Infection During Pregnancy	83 (33.20)	80 (32.39)	0.68
Preeclampsia	55 (22.00)	68 (27.53)	0.19
Antenatal Intrauterine Fetal Death	4 (1.60)	0	0.14

As a result of the analysis, it was found that a statistically significant higher fibrinogen level was observed in the group of pregnant women with FGR having genotypes -455GA *FI* and -455AA *FI* (median – 4.7 g/l, interquartile range – 4.0-5.5 g/l) compared to with women with genotype -455GG *FI* (median 4.5 g/l, interquartile range 4.0-5.1 g/l, p= 0.04).
Other molecular genetic markers showed no significant associations with clinical and laboratory indicators in pregnant women.

Discussion

Our study showed that the genetic variants -455GA *FI* and -455AA *FI* are associated with an increased level of fibrinogen in pregnant women with fetal growth retardation.
Fibrinogen is a plasma globulin consisting of three pairs of identical polypeptide chains: Aα-, Bβ- and γ. The sequences of the polypeptide chains of fibrinogen are encoded in three different genes located in the long arm of the chromosome 14.
Several variants of gene polymorphism have been found, which are in total responsible for fluctuations in the level of fibrinogen in the range of 34-50% (Endler et al., 2003). The -455G/A polymorphism is associated with an increase in the level of fibrinogen (Makacariya, Bicadze, 2006). A high level of fibrinogen in the blood can indicate a high risk of thrombosis (Dyatlova et al., 2015; Makacariya, Bicadze, 2006). Hyperfibrinogenemia is observed in hypercoagulation and in the first stage of DIC-syndrome (Dyatlova et al., 2015).
Previously, researchers also established a relationship of polymorphism -455 G/A *FI* with a higher plasma fibrinogen level (Humphries et al., 1987; Makacariya, Bicadze, 2006; Yenicesu et al., 2010). On the contrary, in other works opposite results were obtained (Poursadegh Zonouzi et al., 2013).
In our study, were found no significant associations of polymorphic loci 20210G/A *FII*, 1691G/A *FV*, 10976G/A *FVII* with clinico-laboratory parameters in pregnant women with FGR.
In other works, contradictory results were also obtained.
Thus, several studies have shown the connection of polymorphism 10976G/A *FVII* with the level of proconvertin in plasma. The presence of the heterozygous variant 10976GA *FVII* was associated with a decrease in the concentration of factor VII in the blood by about 25%, and in the 10976AA *FVII* homozygote – with a factor concentration decrease of approximately 50% compared to the carriers of the variant 10976GG *FVII* (Makacariya et al., 2006, Seremak-Mrozikiewicz et al., 2009).
Some researchers have identified associations of polymorphisms of 1691G/A *FV* and 20210G/A *FII* with the risk of developing FGR (Martinelli et al., 2001; Dudding, Attia, 2004; Howley et al., 2005); in contrast, (Infante-Rivard et al., 2002).

Thus, the conducted study on the relationship of polymorphic locus -455G/A *FI* with a higher level of fibrinogen in pregnant women with FGR. The elevated fibrinogen level associated with the carriage of genotypes -455GA *FI* and -455AA *FI* can serve as a marker of thrombophilia risk and the development of fetal development retardation syndrome.

The obtained results broaden the concept of the role of hereditary thrombophilia in the development of fetal growth retardation and their use in practical obstetrics.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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