ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The association of polymorphisms of vascular reaction genes with blood pressure levels in pregnant women of Belgorod region.

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ABSTRACT

This paper presents the results of studies of the association of genetic polymorphisms of K198N endothelin 1, G460W alpha-adducin with blood pressure indicators in women during early pregnancy. The study was conducted on a sample of 591 women. Blood pressure was analyzed both prior to pregnancy and during pregnancy on week 37-40. Genotyping of the polymorphisms of endothelin-1 (K198N ET-1) and alpha-adducin (G460W ADD1) was carried out by polymerase chain reaction (PCR). It was determined that the genetic variants 198KK and 198KN ET-1, 460WW ADD1 are associated with the more pronounced blood pressure dynamics in women at fertilization and its high level at the end of pregnancy.

Keywords: Pregnancy, arterial hypertension, genetic polymorphism.

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ISSN: 0975-8585

INTRODUCTION

A particular stage of life of women of reproductive age is pregnancy, characterized by severe hormonal, metabolic and hemodynamic changes. Even in healthy women, pregnancy is a "stress test" for the cardiovascular system, which operates with increased load. This increase in load is due to hyper-metabolism, increased circulating blood volume, development of utero-placental circulation, progressive increase in body weight of a pregnant, and increased production of several hormones (Siliberto et al, 2006; Sokolnikova et al, 2008).

Functional disturbances of the cardiovascular system in pregnant women can lead to a reduction of compensatory functions of the fetus, the development of chronic intrauterine hypoxia and placental insufficiency (Tkacheva, Barabashkina, 2006; Murashko et al, 2008). One of the most dangerous manifestations of the dysfunction of the cardiovascular system during pregnancy is hypertension, which prevalence, according to foreign authors, is 5-15% among pregnant women (Sibai, 2004; Clark et al, 2008), and its incidence in different regions of Russia ranges from 5-30% (Tkacheva, Barabashkina, 2006). Pregnant women suffering from hypertension are prone to the development of complications such as a placental abruption, disseminated intravascular coagulation, cerebral hemorrhage, acute renal failure, and eclampsia (Tkacheva, Barabashkina, 2006). Currently, there is a significant amount of data on the effect of different genetic polymorphisms on changes in the functioning of the cardiovascular system in pregnant women (Wang et al, 2004; Moore et al, 2007). However, the results of research for the associations of the genetic markers prone to the development of pathological manifestations on the part of the cardiovascular system in pregnant women are often quite different results in various populations of the world (Sandrim et al, 2008).

The clinical and genetic studies dealing with the molecular-genetic aspects of changes in the functioning of the cardiovascular system during pregnancy have been conducted in the selected populations in Central Russia (Minushkina, 2008).

MATERIALS AND METHODS

Object of study

We have analyzed the survey results of 591 pregnant women (gestational age of 37-40 weeks, mean age - 27.98 ± 4.50 years, ranging 20-43 years). The sample included Russian individuals, who are natives of Central Black Soil region of Russia and have no relationship with each other. Clinical-laboratory and instrumental examination was carried out on the basis of women's Perinatal Center of St. Joasaph Belgorod Regional Clinical Hospital. All surveyed women underwent the analysis of the blood pressure (systolic, diastolic, and average pulse pressure) before pregnancy (data obtained from the patients' case records) and in late pregnancy (on week 37-40 of gestation). 450 individuals among the surveyed women before pregnancy had normotension, 137 - hypotension, and 80 - hypertension. To assess changes in the level of blood pressure at fertilization, the changed values of systolic (ΔSAD), diastolic (ΔDAD), pulse (ΔPD) and medium (ΔADsr) blood pressure were calculated. 209 pregnant women among 591 patients had a physiological course of gestation, and 382 women had pregnancy complicated by preeclampsia.

We performed the typing of two molecular genetic markers of vasoactive hormones, namely diallel gene loci of endothelin-1 (K198N ET-1) and alpha-adducin (G460W ADD1) in all women. The choice of these systems for the study is due to their possible association with the functioning of the cardiovascular system by virtue of the ability of the studied polymorphisms to modify the expression of the corresponding genes or to lead to changes in the structure of the encoded products (Bianchi et al, 2005; Dosenko et al, 2006).

As the material for the study we used 8-9 ml of venous blood taken from the cubital vein of a proband. Venous blood was sampled in test tubes with a preservative containing 0.5 M EDTA solution (pH=8.0).

Molecular and genetic methods

A genomic DNA was isolated from peripheral blood by the method of phenol-chloroform extraction. Analysis of the investigated loci was carried out by the method of polymerase chain reaction (PCR) of DNA synthesis.

DNA markers genotyping was produced by the method of TagMan probes detection according to RFU values (relative fluorescence unit) of each probe on the thermocycler IQ5 with detecting system in real time. "Bio-Rad IQ5-Standart Edition" program was used for the alleles discrimination.

Statistical methods

The formation of database and the statistical calculations were carried out using the "STATISTICA 6.0" program. Determination of phenotypic and gene frequencies was performed by standard methods (Ghivotovsky, 1984). To assess the compliance of the observed distribution of genotypes with the expected one, based on Hardy-Weinberg equilibrium, we used χ^2 test (Vejr, 1995). In performing multiple comparisons, we used the Bonferroni correction in order to minimize the first kind errors (false positive results) (Rebrova, 2006).

During the study of the relationship of genetic polymorphisms with pathogenetically significant quantitative characters, the nature of the distribution of these characters was initially assessed using the Shapiro-Wilk test (Rebrova, 2006). It was found that the distribution of all the studied quantitative characters (blood pressure level) was not consistent with a normal distribution and, therefore, the median (Me) and interquartile range (Q25-Q75) was used for their description, and for comparative analysis - the Mann-Whitney test with Bonferroni corrections (Rebrova, 2006).

RESULTS

The study of the association of polymorphism of K198N endothelin-1 with the indicators of blood pressure in women during late pregnancy found that women with genotypes 198KK and 198KN are characterized by high values of systolic, diastolic, pulse and mean arterial pressure as compared with individuals homozygous for 198N allele (p=0.03-0.001) (Table 1).

Table 1. The associations of polymorphism of K198N endothelin-1 with the blood pressure indicators in women during late pregnancy, Me (Q25-Q75)

Indicators	Genotypes		p
	198KK+98KN (n=572)	198NN (n=15)	
SBP, mm Hg	135.0 (120.0-145.0)	115.0 (110.0-130.0)	0.001
DBP, mm Hg	85.0 (75.0-90.0)	70.0 (70.0-80.0)	0.001
PP, mm Hg	50.0 (40.0-55.0)	40.0 (40.0-50.0)	0.03
BP_{mean}, mm Hg	103.3 (88.3-110.0)	86.7 (83.3-96.7)	0.0007
ΔBP_{mean}, mm Hg	13.3 (5.0-23.3)	5.0 (0.0-16.7)	0.006
ΔSBP, mm Hg	20.0 (10.0-30.0)	5.0 (0.0-20.0)	0.004
ΔDBP, mm Hg	10.0 (0.0-20.0)	5.0 (0.0-15.0)	0.02

Individuals with genotype 198NN also have lower arterial pressure dynamics at the end of pregnancy (systolic, diastolic, and mean arterial pressure) (p=0.02-0.0007).

The study of the associations of polymorphism of G460W alpha-adducin with blood pressure indicators found that the pregnant women homozygous for 460W allele have higher systolic blood pressure (median 140.0 mm Hg) as compared with women with 460GG and 460GW genotypes (median 130 mm Hg, p=0.05).

DISCUSSION

The aim of our study was to evaluate the role of genetic markers of vasoactive hormones in the changes of the cardiovascular system functioning in women during pregnancy. For this purpose, we evaluated blood pressure levels in women during late pregnancy (on week 37-40), and their changes associated with pregnancy. As a result, it was found that all the studied molecular genetic markers are associated with the

level of blood pressure in pregnant women on week 37-40 and its dynamics during the development of pregnancy.

It was also found that 198KK and 198KN genotypes of the genetic polymorphism K198N ET-1 are associated with higher values of blood pressure in pregnant women on week 37-40 and the expressed blood pressure dynamics during the development of pregnancy. There is a conflicting evidence in the literature on this subject. Some studies show the association of 198N allele with elevated blood pressure and elevated levels of endothelin-1 content in the plasma of pregnant women (Barden et al, 2001), while other studies contain no such characteristics (Treiber et al, 2003). Several authors have identified the influence of both genotype 198KK (Castro et al, 2007) and genotype 198KN (Adámková et al, 2006) on the development of left ventricular hypertrophy in individuals with primary hypertension.

One of the factors affecting the level of blood pressure is the change in Na^+ reabsorption rate in the kidney. Adducin protein consisting of α- and β- (or γ-subunits), due to activation of Na^+-K^+-ATPase performs an inverse sodium reabsorption on the basolateral membrane of epithelial cells of renal tubules, resulting in the increased circulating blood volume and blood pressure levels. We have found that the genotype WW of G460W ADD1 locus is a marker of elevated systolic blood pressure in pregnant women on week 37-40. Other studies also show the relationship between 460W allele and the elevated blood pressure levels (Fava et al, 2007). The mechanism of these associations is believed to be related to changes in adducin-induced sodium excretion in kidney (Bianchi et al, 2007; Manunta et al, 2008). G460W mutation in the gene encoding α-subunit protein reduces the internalization process and endocytosis of molecules Na^+-K^+-ATPhase and consequently increases sodium reabsorption through the basolateral membrane of the renal tubules that eventually leads to an increase in blood pressure levels (Bianchi et al, 2007).

Thus, the results of the study conducted the first time ever on a sample of Russian women born in the Central Black Soil Region of Russia have demonstrated the significant associations of polymorphic markers of the genes of vasoactive hormones with blood pressure levels and their dynamics during the development of pregnancy.

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