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ROLE OF VEGF A GENE POLYMORPHISM IN PREGNANT WOMEN WITH CHRONIC VIRAL HEPATITIS B WITH AND WITHOUT D AGENT

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Abstract. *Vascular endothelial growth factor (VEGF) and its receptors play an important role in regulating angiogenesis. The aim of the study was to investigate the VEGF A gene polymorphism in pregnant women with chronic viral hepatitis B (CVHB) with and without the D agent. A prospective observational study was conducted among 188 pregnant women aged 18 to 45 years. The main group included 67 pregnant women with complicated pregnancy outcomes: 32 with CVHB and the D agent, and 35 without the D agent. The comparison group consisted of 70 pregnant women with uncomplicated pregnancies: 23 with CVHB and the D agent, and 47 without the D agent. The control group included 51 healthy pregnant women without a history of chronic viral hepatitis. The distribution and haplotypes of the -2578 C/A polymorphic locus (rs699947) of the VEGF A gene were studied. Changes in the distribution of the studied genotype were associated with the risk of pregnancy complications: the CA genotype demonstrated a protective role in uncomplicated pregnancies in women with CVHB with and without the D agent, while the AA genotype, on the contrary, was associated with an increased risk of pregnancy complications. The reduced frequency of the heterozygous CA genotype and the predominance of the AA genotype among pregnant women indicated a higher probability of adverse pregnancy outcomes.*

Keywords: *D-agent, chronic viral hepatitis B, pregnant women, complicated pregnancy, VEGF A, gene polymorphism, C/A genotype.*

Introduction. Each human genome, except in monozygotic twins, is unique. Population, race, and more importantly, individual genomic characteristics differ not only due to variations in exons (coding regions) but also in non-coding regions (introns, intergenic intervals) and are associated with genetic polymorphism (GP). Polymorphisms are observed in all human genes [1].

Vascular endothelial growth factor (VEGF) and its receptors play a key role in regulating angiogenesis. The VEGF family includes several factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E (identified in Orf virus), and placental growth factor (PlGF). VEGF-A, VEGF-B, and PlGF are the main regulators of blood vessel growth, while VEGF-C and VEGF-D are necessary for lymphatic vessel formation. VEGF-A, also referred to simply as VEGF, is one of the most well-studied factors in angiogenesis [3].

Normal pregnancy depends on angiogenesis during implantation and placental development. Deficiency in these vascular processes can lead to early obstetric complications and pregnancy loss. A number of growth factors stimulate angiogenesis, with VEGF being the most potent among them [7].

During the early stages of pregnancy, VEGF expression is high. This supports oocyte maturation, trophoblast proliferation, and implantation. Altered VEGF expression can lead to fetal development abnormalities and affect placental angiogenesis and uterine blood vessel formation [4].

VEGF as a factor has been studied in many diseases, including oncological conditions. It increases vascular permeability and disrupts vascular integrity, exacerbating hypoxia and promoting tumor cell proliferation and metastasis. Elevated VEGF levels have been found in breast cancer, colorectal cancer, non-small cell lung cancer, renal cell carcinoma, glioblastoma, and other malignancies [5].

Decreased angiogenesis activity is observed in aging, as well as in diseases such as Alzheimer's disease, stroke, and peripheral arterial atherosclerosis. VEGF gene polymorphisms are associated with the risk of ischemic heart disease, hypertension, and myocardial infarction [2].

In the reviewed literature, information was also found regarding the significance of VEGF A in various infectious diseases. For instance, hyperinflammation caused by SARS-CoV-2 infection may enhance activation of the VEGF-A/VEGFR-2 pathway, stimulating angiogenesis, nitric oxide production, increased vascular permeability, and disruption of endothelial cell junctions [6].

Studying the genetic predisposition to diseases associated with endothelial and systemic hemostatic disorders in greater detail allows for a better understanding of the inconsistent data regarding the course and outcomes of chronic viral hepatitis B (CVHB) in pregnant women. Moreover, no information was found in the literature regarding the predictive role of vascular changes caused by chronic infectious processes in the development of pregnancy complications in such patients.

The aim of the study was to investigate VEGF A gene polymorphism in pregnant women with chronic viral hepatitis B (CVHB) with and without the D agent.

Material and methods of research. A prospective case-control study was conducted. A total of 188 pregnant women aged 18 to 45 years (mean age 29.4±0.49 years), all of Uzbek ethnicity, participated in the study. Of these, 137 women who had been treated for chronic viral hepatitis D and B at Tashkent City Infectious Diseases Hospital No. 1 between 2017 and 2021 were included and monitored until delivery.

Based on pregnancy outcomes, the participants were divided into two groups:

The main group consisted of 67 pregnant women with complicated pregnancy outcomes: 32 women (47.8%) with CVHB and the D agent, and 35 women (52.2%) with CVHB without the D agent.

The comparison group included 70 pregnant women with uncomplicated pregnancies: 47 women (67.1%) with CVHB and the D agent, and 23 women (32.9%) without the D agent. There were no statistically significant differences between these groups regarding the etiological agent of the chronic infection ($P>0.05$).

The control group consisted of 51 healthy pregnant women without a history of chronic viral hepatitis.

Blood samples were collected in the morning at 8:00 AM on an empty stomach upon hospital admission. DNA was extracted from peripheral blood samples. Genotyping was performed using PCR with specific primers to identify polymorphisms in the VEGF A gene, specifically focusing on the widespread polymorphism association and haplotypes of the -2578 C/A (rs699947) polymorphic locus.

DNA extraction from peripheral blood and subsequent genetic analysis using real-time PCR were performed using the PROBA-RAPID-GENETIKA reagent kit (catalog number: P-021/4). The identification of the polymorphism association and haplotypes of the -2578 C/A polymorphic locus of the VEGF A gene was conducted using the NP-453-100 DT reagent kit.

The collected data were processed using the "MICROSOFT EXCEL" 2022 (XP) software, employing variation statistics methods. Mean values (M), standard deviations, standard errors (m), and relative values (percentages) were calculated using parametric and nonparametric statistical methods. The statistical significance of differences in quantitative mean values between study groups was assessed using Student's t-test with the probability of error (P). Quantitative changes with a significance level of $P<0.05$ were considered statistically significant.

Correlation analysis between groups was performed using correlation-regression analysis (Pearson's coefficient) with a medical statistical calculator (https://medstatistic.ru/calculators). The statistical significance of differences in qualitative features between groups was assessed using odds ratio (OR) analysis with 95% confidence intervals (CI). When OR was less than 5, Fisher's exact test was used; OR between 5 and 10 was assessed using χ^2 with Yates correction; and OR above 10 was evaluated using χ^2 analysis.

Results and discussion. In the main group of pregnant women with complicated pregnancies, the homozygous CC genotype of the -2578 C/A polymorphism of the VEGF A rs699947 locus was detected in most cases (47.8%), followed by the heterozygous CA genotype in 20.9% and the homozygous AA genotype in 31.3%. According to the Hardy-Weinberg equilibrium (HWE), in an ideal population unaffected by evolutionary, genetic, or other influencing factors, allele and genotype frequencies should remain constant across generations. This law predicts genotype distribution in a population based on allele frequencies. In the main group, according to HWE, the expected genotype frequencies were as follows: CC – 49.3%; CA – 43.3%; and AA – 7.7%. As shown, the actual frequency of the CC genotype corresponded to the expected value, whereas the CA and AA genotype frequencies differed significantly. Specifically, the actual frequency of the CA genotype (20.9%) was significantly lower than expected (43.3%) (OR=2.714; 95% CI=1.1–6.7; χ^2=0.04658; P<0.05), while the actual frequency of the AA genotype (31.3%) was significantly higher than expected (7.7%) (OR=3.909; 95% CI=1.3–11.9; χ^2=0.02214; P<0.05).

The actual frequencies of the C and A alleles in the main group were 58.2% and 41.8%, respectively, whereas, according to HWE, they were expected to be 70.9% and 29.1%. No statistically significant differences were found in allele distribution between the groups (OR=1.571; 95% CI=0.9–2.8; χ^2=1.24854; P>0.05).

We also examined the distribution of the -2578 C/A genotypes in the comparison group of women with uncomplicated pregnancies. In this group, the majority (52.9%) carried the heterozygous CA genotype, 38.6% carried the homozygous CC genotype, and 8.5% carried the homozygous AA genotype. According to HWE, the expected frequencies in this group were: CC – 38.6%; CA – 47.1%; and AA – 14.3%. Analysis showed that the actual and expected frequencies of the CC genotype matched. The actual frequency of the CA genotype (52.9%) was slightly higher than expected (47.1%), but this difference was not statistically significant (OR=1.210; 95% CI=0.5–2.8; χ^2=0.67020; P>0.05). The actual frequency of the AA genotype (8.5%) was slightly lower than expected (14.3%), without reaching statistical significance (OR=1.583; 95% CI=0.4–6.1; χ^2=0.52525; P>0.05).

In the control group of healthy pregnant women, most carried the CA genotype (49.0%), while 48.1% carried the CC genotype and 7.8% carried the AA genotype. According to HWE, the expected frequencies were: CC – 45.1%; CA – 37.3%; and AA – 17.6%. Analysis showed that the actual and expected frequencies of the CC genotype were nearly identical (P>0.05). The frequency of the CA genotype (49.0%) was higher than expected (37.3%), but the difference was not statistically significant (P>0.05). However, the actual frequency of the AA genotype (7.8%) was significantly lower than the expected value (17.6%) (P<0.05).

When comparing the frequencies of the -2578 C/A genotypes between the main and comparison groups, significant differences were observed in the frequencies of the CA and AA genotypes. In the main group, the CA genotype frequency was significantly lower than in the comparison group (OR=4.80; 95% CI=1.66–3.5; χ^2=9.729; P<0.01), while the AA genotype frequency was significantly higher (OR=4.318; 95% CI=1.3–14.3; χ^2=6.349; P<0.01). No significant differences were observed in the frequency of the CC genotype between these groups (P>0.05). Likewise, no significant differences were found in the distribution of C and A alleles between the groups (OR=1.122; 95% CI=0.6–2.06; χ^2=0.138; P>0.05). In the main group, the frequencies of C and A alleles were 58.2% and 41.8%, respectively, whereas in the comparison group, they were 65.0% and 35.0%.

Comparing the main group with pregnancy complications to the control group of healthy pregnant women, significant differences were again found in the frequencies of the CA and AA genotypes. The frequency of the CA genotype was significantly lower in the main group compared to the control group (OR=3.6; 95% CI=1.6–8.1; P<0.05), whereas the frequency of the AA genotype was significantly higher (OR=5.4; 95% CI=1.7–16.8; P<0.01). No significant differences were observed in genotype frequencies between the comparison group and the control group of healthy pregnant women (P>0.05).

Based on these findings, in order to investigate the causes of adverse pregnancy outcomes, we studied the gene polymorphism responsible for vascular endothelial growth factor (VEGF A), which plays a key role in vasculogenesis. It is known that impaired vasculogenesis in the fetus leads to circulatory disorders and adverse pregnancy outcomes. In most pregnant women with complications, the homozygous CC genotype predominated, in agreement with HWE. However, the actual frequency of the CA genotype was significantly lower than expected (OR=2.714; 95% CI=1.1–6.7; χ^2=0.04658; P<0.05), while the frequency of the AA genotype was significantly higher (OR=3.909; 95% CI=1.3–11.9; χ^2=0.02214; P<0.05). In the comparison group, most women carried the heterozygous CA genotype, and both actual and expected frequencies did not differ significantly (P>0.05). In the main group, the frequency of the CA genotype was significantly lower than in the comparison group (OR=4.80; 95% CI=1.66–3.5; χ^2=9.729; P=0.002), while the AA genotype frequency was significantly higher (OR=4.318; 95% CI=1.3–14.3; χ^2=6.349; P=0.012). When comparing with healthy pregnant women, no significant differences were found between the comparison group and the control group regarding genotype frequencies. However, in the main group with pregnancy complications, the frequency of the AA genotype was significantly higher, and the CA genotype significantly lower, compared to healthy pregnant women.

Therefore, it can be concluded that changes in the distribution of the studied genotypes indicate their association with the risk of pregnancy complications. In chronic viral hepatitis B, both with and without the D agent, the CA genotype acts as a protective factor contributing to an uncomplicated pregnancy, while the AA genotype, conversely, increases the risk of adverse pregnancy outcomes. Thus, the decreased frequency of the heterozygous CA genotype and the increased frequency of the homozygous AA genotype are associated with a higher likelihood of pregnancy complications.

Conclusion. In pregnant women with chronic viral hepatitis B, with and without the D agent, the CA genotype of the -2578 C/A polymorphism plays a protective role in the course of pregnancy, while the AA genotype, on the contrary, is associated with an increased risk of pregnancy complications.

Table 1

Analysis of the Frequency Distribution of Alleles and Genotypes of the VEGF A -2578 C/A Gene in the Study Groups

Allele and Genotype	Comparison Group n = 70 abs. (%)	Main Group n = 67 abs. (%)	Control Group n = 51 abs. (%)	OR (95% CI)	P
CC	27 (38,6)	32 (47,8)	22 (43,1)	* 1,495 (0.6-3.5) ** 0,82 (0,4-1,7) *** 1,2 (0,6-2,5)	*>0,05 **>0,05 ***>0,05
CA	37 (52,9)	14 (20,9)	25 (49,0)	* 4.80 (1.66-3.5) ** 1,2 (0,6-2,4) ***3,6 (1,6-8,1)	*<0.01 **>0,05 ***<0.05
AA	6 (8,5)	21 (31,3)	4 (7,8)	* 4.318 (1.3-14.3) ** 1,1 (0,3-4,1) *** 5,4 (1,7-16,8)	*<0.01 **>0,05 ***<0.01
C	91 (65,0)	78 (58.2)	69 (67,6)	* 1.122 (0.6-2.06) ** 0,9 (0,3-2,8) ***0,83 (0,1-1,3)	*>0,05 **>0,05 ***>0,05
A	49 (35,0)	56 (41,8)	33 (32,4)		

Note:
* – Difference between the comparison and main groups;
** – Difference between the comparison and control groups;
*** – Difference between the main and control groups.

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