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STUDY OF THE ASSOCIATION OF VEGF-A GENE rs2010963 POLYMORPHISM WITH THE DEVELOPMENT OF VARICOSE VEINS OF THE LOWER EXTREMITIES AND PHLEBOTHROMBOSIS

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Abstract:

Study of the role of genetic factors, in particular the significance of polymorphic variants of the VEGF-A gene in the development of varicose veins of the lower extremities (VVLE) and its complications may be a promising direction. The aim of the study was to investigate the association of rs2010963 polymorphism of VEGF-A gene with the development of VVLE and its complicated form with deep vein thrombosis. In patients with uncomplicated and with complicated forms of VVLE, the frequencies of rs2010963 of the VEGF-A gene polymorphism in the main and control samples were studied. Real-time PCR analysis of genomic DNA isolated from peripheral blood was performed. The wild-type C/C genotype was statistically significantly, 4.6-fold, detected with high frequency in the control group compared with the group of patients with venous thrombosis ($\chi^2>3.84$; $p<0.05$; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI:0.03-0.15). At the same time, the heterozygous C/G genotype was statistically significantly, 1.8-fold ($\chi^2>3.84$; $p<0.05$; RR=5.6; 95%CI:2.63-11.90; OR=8.2, 95%CI:3.33-20.26). more frequently detected among patients with venous thromboses than among conditionally healthy persons. The proportion of the mutant heterozygous G/G genotype was statistically significantly more prevalent among patients with VVLE complicated by venous thromboses by a factor of 13.8, compared with the control sample ($\chi^2>3.84$; $p<0.05$; RR=12.5; 95%CI:6.26-25.07; OR=23.6; 95%CI:18.92-211.45). Conclusion. The wild-type C/C polymorphism rs2010963 of the VEGF-A gene had protective properties, whereas the heterozygous C/G genotype and the mutant homozygous G/G genotype are risk markers for VVLE and varicose vein thromboses.

Keywords: rs2010963, VEGF-A gene, VVLE, venous thromboses.

The urgency of the problem of VVLE is due to the fairly wide prevalence of the disease, especially among people in the older age group, and in particular among women. The study of the role of growth factors, in particular the value of the vascular endothelial growth factor, a protein responsible for the growth and proliferation of endothelial cells and vessels, and thus for angiogenesis, may become a promising direction [1,2,3]. This is related to the interest shown in the possible role of polymorphic variants of the VEGF gene in the development of such vascular pathology as VVLE and its complications in the form of phlebothrombosis [4,5].

THE AIM OF THE STUDY.

To study the association of the rs2010963 polymorphism of the VEGF-A gene with the development of VVLE and its complication by venous thrombosis.

MATERIALS AND METHODS.

The main group of patients consisted of 161 patients with a predisposition to the development of VVLE and its complications in the form of varicophlebitis. Of these, 111 patients had an uncomplicated form of VVLE, and 50 patients was complicated by venous thrombosis. The comparison was carried out with the control group, which included 155 conditionally healthy individuals. The distribution of the rs2010963 polymorphism of the VEGF-A gene in the studied groups and subgroups was investigated. Leukocytes were isolated using the AmpliPrime RIBO-prep kit (Interlabservice LLC, Russia) for subsequent PCR studies. Polymorphism was detected using test systems of OOO NPF Litekh (Russia). Amplification was performed using a Real-time thermal cycler "Rotor Gene Q" (Quagen, Germany). For statistical calculations, the application package "OpenEpi 2009, Version 9.3" was used.

RESULTS AND DISCUSSION.

As a result of the study, the frequencies of the distribution of alleles and genotypes of the rs2010963 polymorphism in the VEGF-A gene were studied and the search for an association between the differences in their detection and the incidence of VVLE and their complications in the form of varicophlebitis was carried out.

The comparative analysis showed that the frequency of the C allele was significantly lower in the main group of patients, including patients with uncomplicated VVLE and patients with complicated by venous thrombosis, where the frequency of this allele was 54.3%, compared with the control sample, where it was 85.5% ($\chi2> 3.84$; $p<0.05$; RR=0.5; 95%CI:0.45-0.60; OR=0.2; 95%CI:0.14-0.30) (Table 1 and Fig. 1).

The proportion of the prevalence of the G allele in the main group of patients was 45.6%, which was significantly higher compared to conventionally healthy individuals in the control group, where its prevalence was only 14.5%, which indicates the presence of an associative relationship between the detection of this allele and the presence of VVLE ($\chi^2>3.84$; $p<0.05$; RR=1.9; 95%CI:1.68-2.21; OR=5.0; 95%CI:3.37-7.27) (fig. 1 и tab. 1).

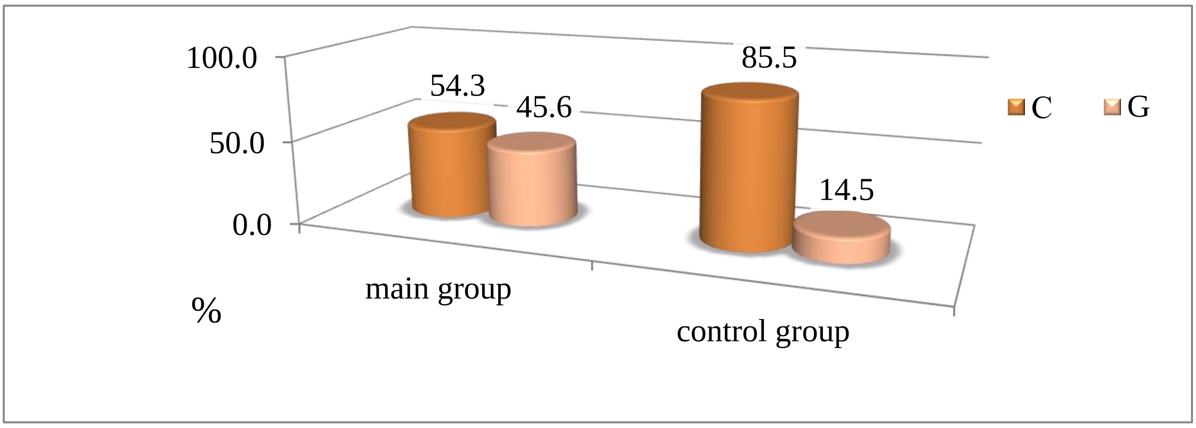

Fig. 1. Frequency of distribution of alleles of rs 2010963 polymorphism VEGF-A gene in main and control groups.

In the main group, uniting patients with uncomplicated VVLE and patients with varicophlebitis, as well as among conventionally healthy individuals in the control sample, the frequencies of distribution of genotypes C/C, C/G, G/G of rs2010963 polymorphism in the VEGF-A gene were studied, and the indicators were in the main group: 31.6%, 45.3% and 23.0%, and in the control group - 74.2%, 22.6% and 3.2% (Fig. 2 and Table 1).

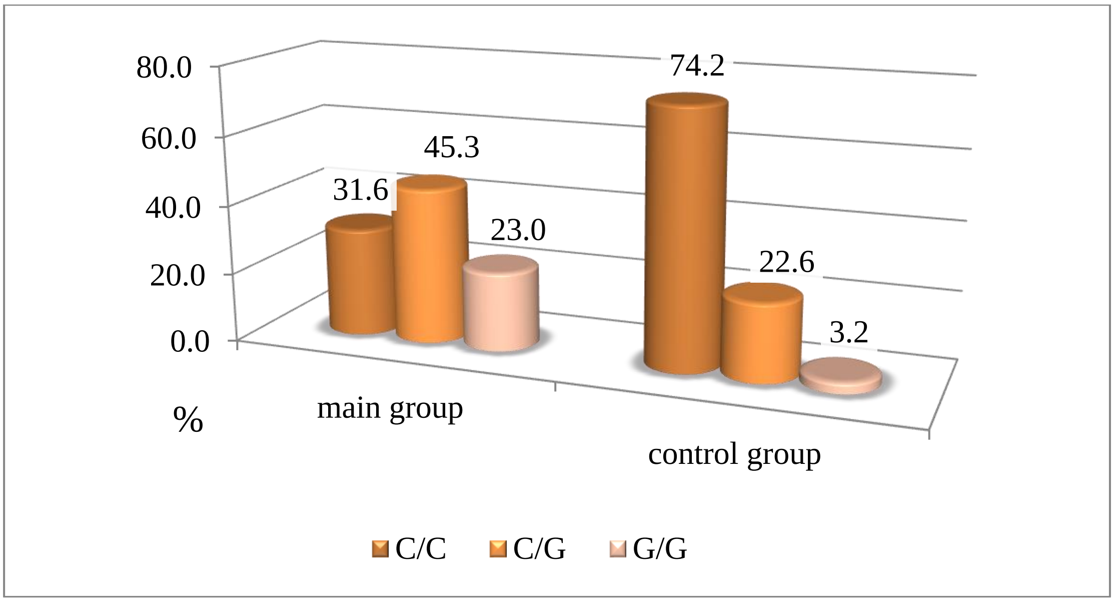

Figure 2. Frequency of distribution of rs2010963 polymorphism genotypes in the VEGF-A gene in the main and control groups

Table 1.
Association between the rs2010963 polymorphism in the VEGF-A gene in the study group of patients and in the control group

Study groups	Alleles and genotypes	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
Main group (n=161)	C	0.5	0.45 - 0.60	0.2	0.14 - 0.30	72.4	0.000001*
	G	1.9	1.68 - 2.21	5.0	3.37 - 7.27		
	C/C	0.4	0.33 - 0.54	0.2	0.10 - 0.26	57.3	0.000001*
	C/G	2.2	1.69 - 2.86	4.7	2.79 - 7.92	35.9	0.000001*
	G/G	2.9	2.22 - 3.70	2.9	2.22 - 3.70	45.2	0.000001*

The proportion of the wild C/C genotype of the rs2010963 polymorphism in the VEGF-A gene in the main group, where its prevalence was 31.6%, was lower than in the control group, in which its proportion was 74.2% (χ2>3.84; p<0.05; RR = 0.4; 95%CI:0.33-0.54; OR=0.2; 95%CI:0.10-0.26).

The concentration of the heterozygous genotype among patients of the main group, C/G was 45.3%, which exceeded its occurrence in the control group, where it was found in 22.6% of cases (χ2>3.84; p<0.05; RR=2.2; 95%CI:1.69-2.86; OR=4.7; 95%CI:2.79-7.92).

The percentage of detection mutant homozygous genotype G/G among patients of the main group was at the level of 23.0% and was higher than the percentage of cases of detection of this genotype in the control sample, which was 3.2% (χ2>3.84; p<0.05; RR=2.9; 95%CI:2.22-3.70; OR=2.9; 95%CI:2.22-3.70) (Fig. 2 and Table 1).

We also studied the prevalence of alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in 111 patients with uncomplicated VVLE, in comparison with the group of conditionally healthy subjects (Table 2, Fig.3 and 4).

It was found that the proportion of the identified C allele of the rs2010963 polymorphic locus of the VEGF-A gene in the main group was 62.6%, which was less than in the control group, where its detection rate was 85.5% (χ2>3.84; p<0.05; RR=0.5; 95%CI:0.45-0.60; OR=0.2; 95%CI:0.14-0.30).

Allele G, on the contrary, prevailed among patients with uncomplicated VVLE, where it was found in 37.4% of cases, which was more frequent than in the control group (14.5%) (χ2>3.84; p<0.05;

RR=1.93; 95%CI:1.68-2.21; OR=5.0; 95%CI:3.37-7.27).

Genotypes C/C, C/G and G/G of the polymorphic locus rs2010963 of the VEGF-A gene were distributed as follows: in the group of patients with VVLE they were: 38.7%, 47.5% and 13.5% versus 74.2%, 22.6% and 3.2% in the control group.

The concentration of the wild C/C genotype in the control group was 74.2%, which is statistically significantly higher than among patients with uncomplicated VVLE, among whom the frequency of its detection was 38.7%, which indicates that this genotype has protective properties in relation to the development of uncomplicated VVLE. ($\chi2>3.84$; $p<0.05$; RR=0.4; 95%CI:0.32-0.58; OR=0.2; 95%CI:0.13-0.37).

The proportion of heterozygous C/G genotype among patients with VVLE was at the level of 47.5%, which is significantly higher ($\chi2>3.84$; $p<0.05$; RR=2.2; 95%CI:1.63-3.01; OR=4.1; 95%CI:2.33-7.04) than among conditionally healthy individuals in the control sample, where its concentration was 22.6%.

The homozygous G/G genotype in the group of patients with VVLE was detected with a frequency of 13.2%, which was significantly higher relative to the control sample, where its concentration was only 3.2%, which indicates its association with the development of an uncomplicated form of VVLE ($\chi2>3.84$; $p<0.05$; RR=2.8; 95%CI:1.92-3.95; OR=8.0; 95%CI:2.75-23.42).

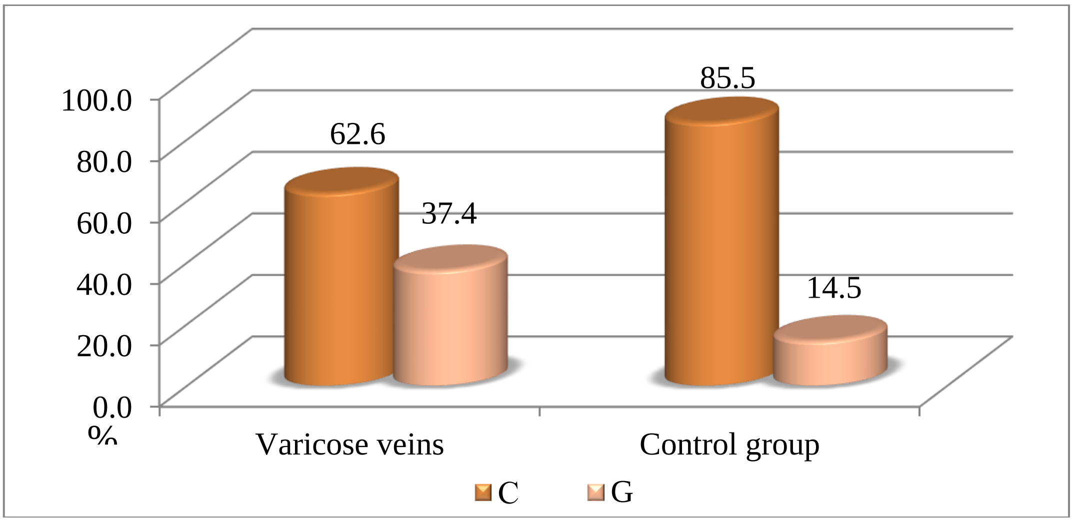

Figure 3. Distribution of alleles of the rs2010963 polymorphism of the VEGF-A gene in the group of patients with uncomplicated VVLE and in the control group

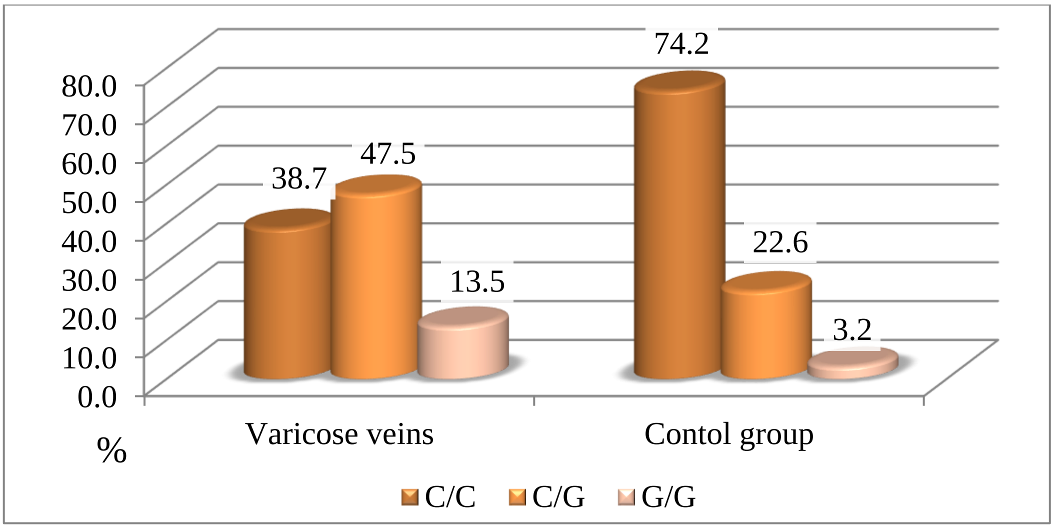

Figure 4. Frequencies of distribution of rs2010963 polymorphism of the VEGF-A gene genotypes in the group of patients with uncomplicated VVLE and in the control group

Table 2.
Associative relationship between alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in groups of patients with uncomplicated VVLE and in the control group

Study groups	Аллели и генотипы	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
Varicose veins (n=111)	C	0.5	0.44 - 0.64	0.3	0.19 - 0.43	37.0	0.000001*
	G	1.9	1.57 - 2.27	3.5	2.32 - 5.34		
	C/C	0.4	0.32 - 0.58	0.2	0.13 - 0.37	33.7	0.000000
	C/G	2.2	1.63 - 3.01	4.1	2.33 - 7.04	25.9	0.000001*
	G/G	2.8	1.92 - 3.95	8.0	2.75 - 23.42	18.5	0.000018*

Also was studied the prevalence of alleles and genotypes of the polymorphic locus rs2010963 of the VEGF-A gene among patients with VVLE complicated by venous thrombosis and in the control group (Table 3 and Fig. 5).

The concentration of the C-allele in the group of patients with varicophlebitis was at the level of 36.0%, which is statistically significantly lower than among conventionally healthy individuals in the control group, where its concentration was 85.5% (χ2>3.84; p<0.05; RR=0.2; 95%CI:0.14-0.29; OR=0.1; 95%CI:0.06-0.16).

The proportion of the G allele, on the contrary, statistically significantly prevailed among patients with venous thrombosis and amounted to 64.0%, which was more than in the control sample, where its occurrence was 14.5% (χ2>3.84; p<0.05; RR=4.9; 95%CI:3.48-6.93; OR=10.5; 95%CI:6.25-17.54).

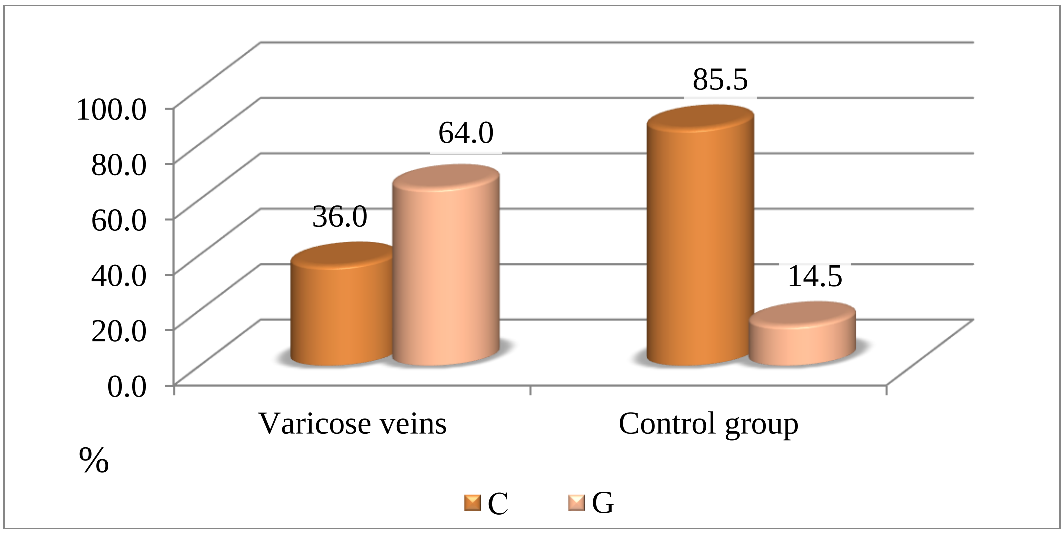

Figure 5. Frequencies of distribution of alleles of polymorphism rs2010963 VEGF-A gene in the group of patients with venous thrombosis and in the control group.

Genotypes C/C, C/G and G/G of the rs2010963 polymorphism of the VEGF-A gene were distributed in the group of patients with varicophlebitis in the following ratio: 16.0%, 40.0%, and 44.0%, and among conventionally healthy individuals in the control group as: 74.2%, 22.6% and 3.2% (Table 3 and Fig. 6).

The proportion of the C/C genotype among patients with VVLE with thrombotic complications in the form of varicophlebitis was 16.0%, which was statistically significantly less than in the control group, where its prevalence was 74.2%, which indicates that it has protective properties in relation to thrombotic complications (χ2>3.84; p<0.05; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI:0.03-0.15).

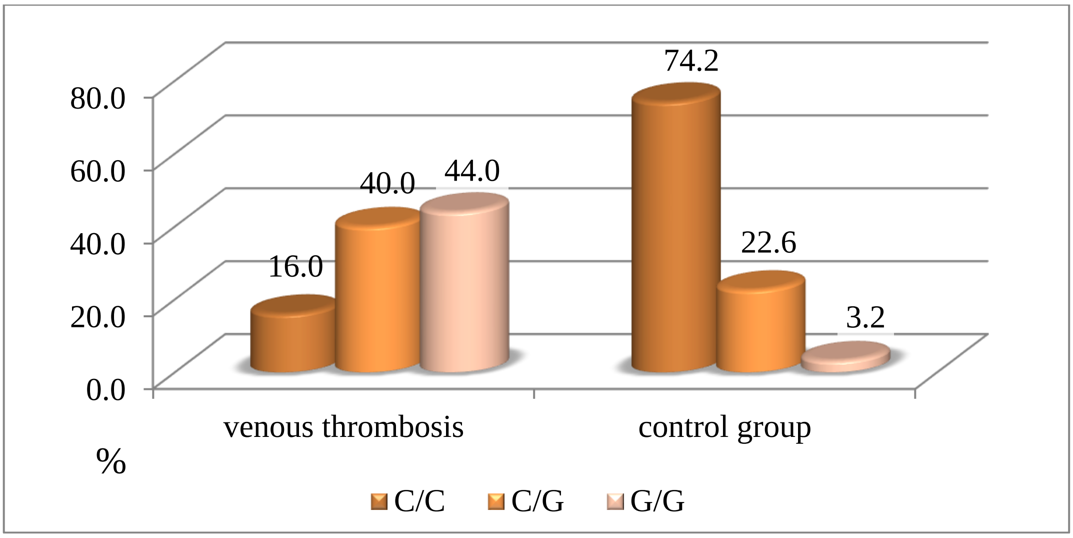

Figure 6. Frequencies of distribution of polymorphism rs2010963 of VEGF-A gene genotypes in the group of patients with venous thrombosis and in the control group

Table 3.
Association between alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in groups of patients with venous thrombosis and in the control group

Study groups	Alleles and genotypes	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
venous thrombosis (n=50)	C	0.2	0.14 - 0.29	0.1	0.06 - 0.16	94.9	0.000001*
	G	4.9	3.48 - 6.93	10.5	6.25 - 17.54		
	C/C	0.1	0.06 - 0.25	0.1	0.03 - 0.15	55.9	0.000001*
	C/G	5.6	2.63 - 11.90	8.2	3.33 - 20.26	25.6	0.000001*
	G/G	13.6	5.4-34.1	23.6	8.2 – 67.4	54.9	< 0.001

The proportion of heterozygous C/G genotype among patients with venous thrombosis was 40.0%, statistically significantly prevailing compared to the control group, where the detection rate was 22.6% (χ2>3.84; p<0.05; RR=5.6; 95%CI:2.63 -11.90; OR=8.2, 95%CI: 3.33-20.26).

The proportion of mutant homozygous genotype G/G among patients with VVLE complicated by thrombotic complications in the form of varicophlebitis was 44.0%, statistically significantly predominant, compared with the control group, where it was detected only in 3.2% of cases (χ2>3.84; p<0.05; RR=12.5; 95%CI:6.26-25.07; OR= 23.6; 95%CI:18.92-211.45).

CONCLUSION.

In general, according to researchers, the rs2010963 polymorphism of the VEGF-A gene is a predictor of various pathologies, including vascular [6,7,8]. However, the role of this polymorphic locus in the occurrence of VVLE and venous thrombosis has not been sufficiently studied [9], and therefore this study was carried out.

During the study of the distribution of rs2010963 polymorphism genotypes in the VEGF-A gene for the presence of an association in the main group of patients and the control sample, it was found that the identification of the heterozygous C/G genotype increases the risk of developing the disease by 2.2 times (χ2=3.84; p<0.05; 95%CI:1.69-2.86), detection of the homozygous mutant G/G genotype increases the risk of developing the disease by almost

2.9 times (χ2=3.84; p<0.05; 95%CI:2.22-3.70) (Table 1).

The results obtained in the course of studies in a group of patients with uncomplicated form of VVLE showed that the detection of the heterozygous C/G genotype increases the risk of developing the disease by 2.2 times (χ2=3.84; p<0.05; 95%CI:1.63-3.01), and the detection of a homozygous mutant genotype G/G increases the risk of developing this form of the disease by 2.8 times (χ2=3.84; p<0.05; 95%CI:1.92 - 3.95) (Table 2).

At the same time, a study in the group of patients with VVLE complicated by varicophlebitis showed that the identification of the heterozygous C/G genotype increases the risk of developing a form of the disease complicated by venous thrombosis by more than 5.6 times (χ2=3.84; p<0.05; 95%CI:2.63-11.90), and the detection of the homozygous mutant genotype G/G - more than 13.6 times (χ2=3.84; p<0.05; 95%CI:5.4-34.1) (Table 3).

In general, our study indicated the presence of a protective role of the C/C genotype (χ2>3.84; p<0.05; RR=0.4; 95%CI:0.33-0.54; OR=0.2; 95%CI:0.10-0.26), in contrast from C/G and G/G genotypes, more often detected among patients with both complicated and uncomplicated forms of VVLE (χ2>3.84; p<0.05; RR=2.2; 95%CI:1.69-2.86; OR=4.7; 95%CI:2.79-7.92) and (χ2>3.84; p<0.05; 95%CI:2.22-3.70; OR=2.9; 95%CI:2.22-3.70).

The obtained results also indicate the protective properties of the wild C/C genotype in relation to VVLE (χ2>3.84; p<0.05; RR=0.4; 95%CI:0.32-0.58; OR=0.2; 95%CI:0.13-0.37), in the difference from which the heterozygous C/G genotype and the mutant homozygous G/G genotype can be considered risk factors for the uncomplicated form of VVLE (χ2>3.84; p<0.05; RR=2.2; 95%CI:1.63-3.01; OR=4.1; 95%CI:2.33-7.04) and (χ2>3.84; p<0.05; RR=2.8; 95%CI:1.92-3.95; OR=8.0; 95%CI:2.75-23.42), respectively.

The study showed that the wild homozygous C/C genotype exhibited protective properties in relation to the development of varicophlebitis (χ2>3.84; p<0.05; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI:0.03-0.15), and the heterozygous C/G genotype and the mutant homozygous G/G genotype acted as genetic markers of the risk of thrombotic complications in patients with VVLE (χ2>3.84; p<0.05; RR=5.6; 95%CI:2.6-11.9; OR=8.2, 95%CI:3.3-20.3) and (χ2>3.84; p<0.05; RR=13.6; 95%CI:5.4-34.1; OR=23.6; 95%CI:8.2-67.4).

Conclusions. The wild C/C genotype of the rs2010963 polymorphism of the VEGF-A gene has protective properties, and the heterozygous C/G genotype and the mutant homozygous G/G genotype are markers of the risk of VVLE and venous thrombosis.

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