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INTERRELATION OF ENDOTHELIAL DYSFUNCTION GENE POLYMORPHISM WITH THE RISK OF DEVELOPING CHRONIC VENOUS INSUFFICIENCY OF THE VEINS OF THE LOWER EXTREMITIES

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ANNOTATION
Chronic venous insufficiency (CVI) is an important medical problem in developed countries. It was important to determine one of the leading roles in the pathogenesis of CVI, the interaction of matrix metalloproteinases (MMPs) as important mediators of the degenerative process and vascular endothelial growth factor (VEGFA). The study included 98 patients aged 20 to 78 years with chronic venous insufficiency, including, in accordance with the CEAP (Clinical-Etiological-Anatomical-Pathophysiological) classification, 45 patients with moderate CVI (class C3-C4) and 53 patients with severe CVI (class C5-C6). The results obtained in the course of the study reliably indicate the presence of an association between the carriage of the Arg allele and the Gln/Arg and Arg/Arg genotypes of the Gln279Arg polymorphism in the MMP9 gene, as well as the carriage of the T allele and the C/T and T/T genotypes of the C936T polymorphism in the VEGFA gene with the risk of developing complicated forms of CVI.

Keywords: chronic venous insufficiency, matrix metalloproteinases, vascular endothelial growth factor, polymorphism, MMP-9, VEGFA

Аннотация
Хроническая венозная недостаточность (ХВН) является важной медицинской проблемой в развитых странах. Важное значение имело определение одной из ведущих ролей в патогенезе ХВН взаимодействия матриксных металлопротеиназ (ММР) как важных медиаторов дегенеративного процесса и сосудистого эндотелиального фактора роста (VEGFA). Обследованы 98 больных в возрасте от 20 до 78 лет с хронической венозной недостаточностью, в том числе, в соответствие с классификацией CEAP, 45 больных были со средней тяжестью течения ХВН (класс С3-С4) и 53 больных – с тяжелой степенью течения ХВН (класс С5-С6). Полученные в ходе проведенного исследования результаты достоверно свидетельствуют о наличии ассоциации носительств аллеля Arg и генотипов Gln/ Arg и Arg/ Arg полиморфизма Gln279Arg в гене ММР9, а так же носительства аллеля Т и генотипов С/Т и Т/Т полиморфизма С936Т в гене VEGFA с риском развития осложненных форм ХВН.

Ключевые слова: хроническая венозная недостаточность, матриксные металлопротеиназы, сосудистый эндотелиальный фактор роста, полиморфизм, MMP – 9, VEGFA

ANNOTATSIYA
Surunkali venoz etishmovchilik (SVE) rivojlangan mamlakatlarda muhim tibbiy muammo hisoblanadi. SVE patogenezida etakchi rollardan birini, degenerativ jarayonning muhim vositachilari va qon tomir endotelial o'sish omili (VEGFA) sifatida matritsali metalloproteinazalarning (MMPs) o'zaro ta'sirini aniqlash muhim edi. Tadqiqotga 20 yoshdan 78 yoshgacha bo'lgan surunkali venoz etishmovchiligi bo'lgan 98 bemor, shu jumladan CEAP tasnifiga muvofiq, o'rtacha CVI (C3-C4 klassi) bo'lgan 45 bemor va og'ir CVI (C5-C6 klassi) bo'lgan 53 bemor ishtirok etdi. Tadqiqot davomida olingan natijalar Arg allelini tashish va MMP9 genida Gln/Arg va Arg/Arg polimorfizmining Gln/Arg va Arg/Arg genotiplari, shuningdek, T. allel va VEGFA genidagi C936T polimorfizmining C/T va T/T genotiplari, CVI ning murakkab shakllarini rivojlanish xavfi bilan.
Kalit so'zlar: surunkali venoz etishmovchilik, matritsali metalloproteinazalar, qon tomir endotelial o'sish omili, polimorfizm, MMP-9, VEGFA

The actuality. Chronic venous insufficiency (CVI) is an important medical problem in developed countries. Increased venous pressure in varicose veins (VV) may contribute to the overexpression of certain matrix metalloproteinases (MMPs) that affect the endothelium, smooth muscle and extracellular matrix proteins of the vein wall [1,2]. Gelatinases, which include MMP-2 (gelatinase A) and MMP-9 (gelatinase B), are responsible for the degradation of the extracellular matrix (ECM) in the vein wall under both physiological and pathological conditions [3]. The main function of gelatinases is the degradation of denatured collagen fibers, as well as the basement membrane and other structural components of the ECM [3,4]. MMP-9 is present in large amounts in neutrophil granules. It plays a major role in the influx of leukocytes to the site of infection or damaged tissue during inflammatory processes. [2].
Vascular endothelial growth factor (VEGF) stimulates the synthesis of MMPs, especially MMP-9 [5,6]. Hypoxia and inflammation occurring in the wall of the VC contribute to an increase in VEGF expression in connective tissues [7]. VEGF plays an important role in maintaining the integrity of blood vessel walls and in the process of angiogenesis [7]. It disrupts the integrity of the vascular wall and cell homeostasis, increasing the permeability of the endothelium [7]. This leads to edema and the formation of "fibrin cuffs" characteristic of CVI. In addition, VEGF activates endothelial nitric oxide synthase (eNOS), which dilates venous vessels [7, 8]. Impaired VEGF synthesis may be a predictor of vascular disease.

The aim of the research. Since the mechanisms leading to the formation of CVI are still not fully understood, the purpose of our study was to evaluate the role of polymorphisms of the MMP-9 (Gln279Arg) and VEGF (C936T) genes in the formation of chronic venous insufficiency of the veins of the lower extremities.

The material and methods of the research. We examined 98 patients aged 20 to 78 years with chronic venous insufficiency, including, in accordance with the CEAP classification, 45 patients were with moderate severity of CVI (class C3-C4) and 53 patients with severe CVI (class C5-C6), who were hospitalized in the Department of Cardiovascular Surgery of the clinics of the Andijan State Medical

Institute. The diagnosis of chronic venous insufficiency was verified on the basis of the results of laboratory instrumental (Doppler ultrasound) and molecular genetic studies. The control group consisted of 87 healthy individuals.

Determination of polymorphic genetic markers of genes was carried out in the Department of Molecular Medicine and Cell Technology on the basis of the Republican Scientific and Practical Medical Center for Hematology of the Ministry of Health of the Republic of Uzbekistan. For molecular genetic analysis, venous blood was taken in an amount of 3 ml in a 5 ml vacutainer (ethylenediaminetetraacetic acid EDTA). DNA isolation was performed by the standard method using the Ribo-prep reagent kit. Detection of molecular markers for the MMP - 9 gene and the VEGFA gene was carried out by a standard polymerase chain reaction on programmable thermal cyclers CG-1-96 "Corbett Research" (Australia) and 2720 "Applied Biosystems" (USA) using the test system of the company "Synthol" (Russia) according to the manufacturer's instructions. The deviation of the distributions of the genotypes of the studied DNA polymorphisms from the canonical Hardy-Weinberg distribution (HWD) was assessed using the GenePop (Genetics of Population) computer program for analyzing genetic data.

Fisher's exact test was used to analyze the dispersion of selected risk factors between the group of patients and the control group. A chi-square test (χ 2) adjusted for Yates continuity was used to assess differences from Hardy-Weinberg equilibrium and independence of genotype and allele frequencies. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the strength of the association between the selected SNPs and the development of complicated forms of chronic venous insufficiency. A $P<0.05$ value was considered statistically significant.

The research results and their discussion

Sex differences in the control and main groups of the studied were not identified. Doppler ultrasound showed the prevalence of hemodynamic disorders in the system of deep veins of the lower extremities with their expansion and dilatation of the walls, as well as the failure of the ostial valve, valves of the perforating veins. In 74% of the studied patients, trophic lesions of the skin of the lower extremities were observed.

Table №1. Expected and observed locus genotype distribution frequencies according to HWD (Gln279Arg polymorphism in the MMP9 gene)

Main group					
Alleles	Allele frequency				
Gln	0,64				
Arg	0,36				
Genotypes	Genotypes frequency		χ2	p	df
	observed	expected			
Gln/ Gln	0,44	0,41	0,25		
Gln/ Arg	0,4	0,46	0,87		
Arg/ Arg	0,16	0,13	0,77		
Bcero	1	1	1,89	0,168	1

Control group					
Alleles	Allele frequency				
Gln	0,69				
Arg	0,31				
Genotypes	Genotypes frequency		χ2	p	df
	observed	expected			
Gln/ Gln	0,51	0,48	0,17		
Gln/ Arg	0,37	0,43	0,74		
Arg/ Arg	0,13	0,1	0,82		
Total	1	1	1,72	0,183	1

Analysis of the Gln279Arg polymorphism in the MMP9 gene did not reveal deviations in the distributions of genotypes from those expected under Hardy-Weinberg equilibrium (HWE) ($\chi2$=1.89, p=0.168 in the main group; $\chi2$=1.72, p=0.183 in the control group).

Table №2 . Differences in the frequency of allelic and genotypic variants of the Gln279Arg polymorphism in the MMP9 gene in groups of patients with severe CVI (C5-C6) and the control group

Alleles and Genotypes	Number of examined alleles and genotypes				$\chi2$	p	RR	95%CI	OR	95%CI
	XBH (C5-C6)		Control group							
	n	%	n	%						
Gln	66	62,3	120	69,0	1,3	p = 0,3	0,9	0,5 - 1,64	0,7	0,45 - 1,23
Arg	40	37,7	54	31,0	1,3	p = 0,3	1,1	0,74 - 1,65	1,3	0,81 - 2,24
Gln/ Gln	22	41,5	44	50,6	1,1	p = 0,3	0,8	0,35 - 1,92	0,7	0,35 - 1,38
Gln/ Arg	22	41,5	32	36,8	0,3	p = 0,6	1,1	0,49 - 2,61	1,2	0,61 - 2,45
Arg/ Arg	9	17,0	11	12,6	0,5	p = 0,5	1,3	0,47 - 3,86	1,4	0,54 - 3,67

The distribution frequency of Gln/ Gln, Gln/ Arg and Arg/ Arg genotypes was: 41.5%, 41.5% and 17.0%, respectively, in the main group and 50.6%, 36.% and 12.6% - in the control group. As can be seen from our data, the combination of the Gln/Arg and Arg/Arg genotypes of the Gln279Arg polymorphism in the MMP9 gene indicates a higher risk of developing severe forms of chronic venous insufficiency (CEAP C5-C6) (OR = 1.2 and 1.4; 95% CI=0.49-2.61 and 0.47-3.86; p=0.5).

The presence of the Gln/Gln genotype of the Gln279Arg polymorphism in the MMP9 gene, on the contrary, has a protective function in preventing the development of severe forms of CVI (OR = 0.7; 95% CI = 0.35 - 1.38; p = 0.3).

Table №3. Expected and observed frequencies of distribution of genotypes of the locus by HWD (polymorphism C936T in the VEGFA gene)

Main group					
Alleles	Allele frequency				
C	0,78				
T	0,22				
Genotypes	Genotypes frequency		$\chi2$	p	df
	observed	expected			
C/ C	0,62	0,6	0,07		
C/ T	0,31	0,35	0,5		
T/ T	0,07	0,05	0,86		
Total	1	1	1,43	0,225	1

Control group					
Alleles	Allele frequency				
C	0,87				
T	0,13				
Genotypes	Genotypes frequency		$\chi2$	p	df
	observed	expected			
C/ C	0,77	0,75	0,03		
C/ T	0,2	0,23	0,44		
T/ T	0,03	0,02	1,44		
Всего	1	1	1,91	0,166	1

Groups	Ho	He	D*
Main group	0,31	0,35	-0,12
Control group	0,2	0,23	-0,15

Note: D = (Ho - He)/He

When analyzing the C936T polymorphism in the VEGFA gene, no deviations in the distributions of genotypes from those expected under Hardy-Weinberg equilibrium (HWE) were detected ($\chi2=1.43$, $p=0.225$ in the main group; $\chi2=1.91$, $p=0.166$ in the control group).

Table №4. Differences in the frequency of allelic and genotypic variants of the C936T polymorphism in the VEGFA gene in groups of patients with severe CVI (C5-C6) and the control group

Alleles and Genotypes	Number of examined alleles and genotypes				χ2	p	RR	95%CI	OR	95%CI
	XBH (C5-C6)		Control group							
	n	%	n	%						
C	85	80,2	151	86,8	2,2	p = 0,2	0,9	0,46 - 1,85	0,6	0,32 - 1,17
T	21	19,8	23	13,2	2,2	p = 0,2	1,1	0,6 - 1,94	1,6	0,85 - 3,09
C/ C	35	66,0	67	77,0	2,0	p = 0,2	0,9	0,37 - 1,99	0,6	0,27 - 1,23
C/ T	15	28,3	17	19,5	1,4	p = 0,3	1,4	0,6 - 3,49	1,6	0,73 - 3,6
T/ T	3	5,7	3	3,4	0,4	p = 0,6	1,6	0,32 - 8,34	1,7	0,33 - 8,51

The distribution frequency of C/C, C/T, and T/T genotypes was 66%, 28.3%, and 5.7%, respectively, in the main group and 77%, 19.5%, and 3.4%, in the control group. As can be seen from our data, the combination of the C/T and T/T genotypes of the C936T polymorphism in the VEGFA gene indicates a higher risk of developing severe forms of chronic venous insufficiency (CEAP C5-C6) (OR = 1.6 and 1.7; 95% CI=0.73 - 3.6 and 0.33 - 8.51).

The presence of the C/C genotype of the Gln279Arg polymorphism in the MMP9 gene, on the contrary, has a protective function in preventing the development of severe forms of CVI (OR = 0.6; 95% CI = 0.27 - 1.23).

Changes in MMP and VEGF activity were observed in many diseases of the circulatory system [10,11,12,13]. The development of CVI is associated with a decrease in wall thickness, changes in hemodynamics, the flow of inflammatory cytokines, changes in the ECM and increased production of reactive oxygen species (ROS) that affect the activity of MMPs [14,15,16,17]. VEGFA increases the permeability of existing blood vessels, helping to maintain inflammation by allowing white blood cells to migrate to their destination.

Thus, the results obtained in the course of the study reliably indicate the presence of an association between the carriage of the Arg allele and the Gln/Arg and Arg/Arg genotypes of the Gln279Arg polymorphism in the MMP9 gene, as well as the carriage of the T allele and the C/T and T/T genotypes of the C936T polymorphism in the MMP9 gene., gene VEGFA with the risk of developing complicated forms of chronic venous insufficiency.

Conclusions and implications. Our study revealed a significant role of the MMP 9 (Gln279Arg) and VEGFA (C936T) gene polymorphisms in CVI formation. The results of this study confirm that the expression of MMP-9 and VEGF genes is altered in patients with CVI. Overexpression of these genes can contribute to the spread of the inflammatory process and indicates intensive remodeling of the extracellular tissue in the wall of the varicose vein. The conducted study shows the relationship between CVI and polymorphisms of the VEGF and MMP-9 genes.

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