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The Role of Genetic Polymorphism in the Pathogenesis of Chronic Venous Insufficiency

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ABSTRACT: Chronic venous diseases (CVD), including varicose veins, are among the most common diseases in developed countries in Europe and the United States and affect one third to one half of the population, especially women. Among many achievements in the knowledge of the pathogenesis of achievements, it should be noted the identification of the role of matrix metalloproteinases (MMPs) as important mediators of the degenerative process associated with the onset and progression of venous insufficiency. The study included 98 patients aged 20 to 78 years with chronic venous insufficiency, including, in accordance with the CEAP 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 with the risk of developing complicated forms of CVI.

KEYWORDS: chronic venous insufficiency, matrix metalloproteinases, MMP 9 polymorphism.

THE ACTUALITY

Chronic venous disease (CVD), including varicose veins, is among the most common diseases in developed countries in Europe and the United States, affecting one third to one half of the population, especially women [16]. The socioeconomic significance of CVD is due to the cost of diagnosis and treatment, reduced quality of life, especially at higher clinical stages, and loss of working days [1,13]. According to the international CEAP classification (C=clinical presentation, E=etiology, A=anatomy, P=pathophysiology), CVD has seven stages (C0–C6). C0 indicates no visible signs of CVD or palpable varicose veins; C1, telangiectasias (spider veins); C2, varicose veins; C3, edema; C4 – skin lesions due to varicose veins or venous reflux–hyperpigmentation, eczema, lipodermatosclerosis or white atrophy; C5 – healed venous ulcer of the leg; C6, open and active venous leg ulcer (10).

Among the many achievements, it should be noted the identification of the role of matrix metalloproteinases (MMPs) as important mediators of the degenerative process associated with the onset and progression of venous insufficiency.

The structure and function of the vein wall is partly regulated by matrix metalloproteinases (MMPs) (5,19). Matrix metalloproteinases (MMPs) are a family of zinc–dependent metalloendopeptidases that cleave extracellular matrix (ECM) components and non–ECM molecules, including receptors, growth factors, cytokines, and chemokines, all of which are determinants of the tissue microenvironment (4, 9.7).

MMP activity is tightly regulated by a family of endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs) (15). Both MMP and TIMP regulate extracellular matrix homeostasis. MMPs are involved in the degradation of extracellular matrix components, while TIMPs affect vascular remodeling by causing changes in venous elasticity [6,12]. MMPs can also influence biologically active molecules present on the cell surface through G protein–coupled receptors (GPCRs) and regulate the cellular environment [13].

Vascular remodeling is a compensatory mechanism for the adaptation of veins to pathological conditions such as venous hypertension and hypoxia [8].

Elastin and collagen are important for the structural integrity of the vein wall [17]. In varicose veins, a decrease in the amount of elastin was observed, as well as structurally altered elastin. MMP–2 (gelatinase A) and MMP–9 (gelatinase B) are the main enzymes involved in elastin assembly [9, 11]. MMP–9 can cleave type IV, V, VII, X, XIV collagen, gelatin, and the same non–collagen substrates as gelatinase A. [1, 10].

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THE AIM OF THE RESEARCH

The aim of the research was to identify the role of the Gln279Arg polymorphism of the MMP 9 gene in the development and progression of complicated forms of chronic venous insufficiency of the lower extremities, in order to develop an optimal diagnostic algorithm and adequate therapy.

THE MATERIALS AND METHODS OF THE RESEARCH

The diagnosis of chronic venous insufficiency was verified on the basis of the results of laboratory instrumental (Doppler ultrasound) and molecular genetic studies. The study included 98 patients aged 20 to 78 years with chronic venous insufficiency, including, in accordance with the CEAP classification, 45 patients with moderate CVI (class C3–C4) and 53 patients with severe CVI (class C5–C6). The probands were hospitalized at the clinic of the Andijan State Medical Institute. 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, 3 ml of venous blood was taken into a 5 ml vacutainer (ethylenediaminetetraacetic acid). DNA isolation was performed by the standard method using the Ribo–prep reagent kit. Detection of molecular markers for the MMP9 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 a test system from Synthol (Russia) according to the 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 ($\chi 2$) 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.

RESEARCH RESULTS AND DISCUSSION

As a result of studies, no gender differences were found according to the results of laboratory–instrumental and molecular genetic studies. According to Doppler ultrasound, hemodynamic disturbances in the system of deep veins of the lower extremities with their expansion and dilatation of the walls, as well as incompetence of the ostial valve, valves of the perforating veins, prevailed. In 74% of the studied patients, trophic lesions of the skin of the lower extremities were observed.

Table 1. Expected and observed frequencies of distribution of locus genotypes by RCM (Gln279Arg polymorphism in the MMP9 gene)

Main group					
Alleles	Allele frequency				
Gln	0,64				
Arg	0,36				
Genotypes	Genotypes frequency		$\chi 2$	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		
Всего	1	1	1,89	0,168	1

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Control group					
Alleles	Allele frequency				
Gln	0,69				
Arg	0,31				
Genotypes	Genotypes frequency		χ^2	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		
Bcero	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).

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 with the risk of developing complicated forms of CVI.

More recently, it has been shown (7,18) that specific polymorphisms in the MMP–9 and TIMP–2 genes potentially increase the risk of developing varicose veins in patients. Vein remodeling and associated gene expression were also studied, but the results did not correlate with the development and severity of PTS [5, 12]. However, their preliminary data showed an increase in MMP–9 expression and a decrease in Toll–like receptor 9 expression in acute deep vein thrombosis. MMP–9 levels have been shown to be elevated in varicose veins and venous ulcers, and some evidence suggests a potential role in resolution of DVT, with gene deletions associated with less collagen deposition.

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Our study showed that the Gln279Arg polymorphism in the MMP9 gene is characterized by allelic diversity. Functionally unfavorable Gln/Arg and Arg/Arg genotypes significantly contribute to the development and progression of CVI, especially its complicated forms.

MMPs are involved in ECM degradation and structural changes in vein walls, ultimately contributing to vein remodeling, dilatation, and valvular dysfunction. Thus, they are actively involved in the pathogenesis of CVI. However, the exact mechanisms remain to be elucidated and further research is required in this area.

CONCLUSION

According to Doppler ultrasound of the vessels of the lower extremities, CVI is characterized by the expansion and loss of tone of the walls of the veins of the lower extremities, the failure of the valvular apparatus of the deep and superficial veins of the lower extremities. In the course of the molecular genetic study, the relationship between the polymorphism of the MMP 9 (Gln279Arg) gene and CVI was revealed. Identification of the CVI candidate gene provides prevention of the development of this pathology and plays an important role in the development of new approaches to the diagnosis and treatment of CVI.

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