

Research of the Involvement of Rs2276109 Polymorphism of the Mmp-12 Gene in the Development of Varicose Vein Disease of the Lower Extremities and Deep Vein Thrombosis of the Lower Extremities

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Annotation: The presence of the rs2276109 polymorphism of the MMP12 gene in the genotype may be a marker of a hereditary predisposition to the development of rearrangements of the structure of the venous walls and, accordingly, a predictor of the development of varicose veins of the lower extremities (VVLE).

Objective. To reveal the association of the rs2276109 polymorphism of the MMP12 gene with the development of varicose veins of the lower extremities (VVLE) and deep vein thrombosis of the lower extremities (DVTLE).

Materials and methods. The studies were carried out in a group consisting of patients with a hereditary predisposition to VVLE and with phlebothrombosis (n=161). The frequency of detection of the rs2276109 polymorphism in the MMP12 gene was studied in all the subjects of the main and control samples.

Key words: polymorphism, rs2276109 (A-82G), MMP12 gene (matrix metalloproteinase 12), varicose veins of the lower extremities (VVLE).

Results. In the course of the research, statistically insignificant differences were found in the frequency of distribution of allelic and genotypic variants of the rs2276109 polymorphism of the MMP12 gene in the patient group and the control sample. Statistical differences in the groups of patients with venous thrombosis and controls are insignificant. In addition, despite the high level of chance of detecting an unfavorable G/G genotype among patients compared to conventionally healthy individuals (OR=3.0), the differences in the frequency of occurrence of this locus in these comparative samples turned out to be statistically insignificant (2.0% versus 0.6%, respectively $\chi^2=0.6$; $p=0.4$; OR=3.0; 95% CI:0.18-48.23).

Conclusion. The rs7123600 polymorphism of the MMP-12 gene cannot serve as an independent, early marker in the development of structural changes in the vein wall and varicose and venous diseases of the lower extremities.

Varicose veins of the lower extremities (VVLE) is very widespread among people of a certain age category over 40 years old, especially among women. According to international data, the incidence of VVLE can reach up to 40% among the population [1]. According to Russian researchers, this indicator slightly exceeds 30% of the total population [2]. The morbidity structure is dominated by women (63% of women and 37% of men) [3].

Unfortunately, in Uzbekistan and in the Central Asian region, there is no information about large-scale research in this direction. There are only some very limited studies of this pathology, and therefore the epidemiological data is insufficient. The pathogenetic basis of VVLE is a genetically determined violation of metabolism in the extracellular matrix, namely in the regulation of individual components, the degradation of which occurs as a result of proteolytic enzymes, for the synthesis of which endotheliocytes and macrophages are responsible [4, 5]. These enzymes include matrix metalloproteinases (MMPs), which are initially secreted in an inactive form. The regulation of their expression directly depends on the homeostasis of the extracellular matrix (ECM). One of the known metalloproteinases, MMP12, has already been shown to relate the rs2276109 (A-82G) polymorphism of the MMP12 gene to the development of oncopathology, endometriosis, and coronary heart disease (IHD) [6, 7, 8, 9, 10, 11].

The release by macrophages-induced matrix metalloproteinase 12 (MMP12) leads to the destruction of several components of the ECM, which facilitates the penetration of macrophages into the site of tissue damage [12,13]. The developing local inflammatory reaction leads to the activation of free radical oxidation, and the release of protease enzymes leading to the destruction of collagen fibers of the vein wall. This, in turn, leads to an increase in the activity of the proliferative activity of smooth muscle myocytes. However, newly synthesized young myocytes synthesize in a much larger amount the components of the extracellular matrix and vice versa in a smaller amount of contractile elements, which affects both the morphological structure and the functional capabilities of the venous wall. Morphologically, this leads to thickening and disorganization of the connective tissue of the venous wall, which reduces its ability to perform the frame function and reduce the overall contractility of the vein [14]. As a result, these changes lead to pathological restructuring and varicose veins [15].

The presence of the rs2276109 (A-82G) polymorphism of the MMP12 gene in the genotype may be a marker of a hereditary predisposition to the development of rearrangements of the structure of venous walls and, accordingly, a predictor of the development of VVLE.

Objective. To reveal the association of the rs2276109 polymorphism of the MMP12 gene with the development of varicose veins of the lower extremities (VVLE) and deep vein thrombosis of the lower extremities (DVTL).

Materials and methods. The study involved 316 people, including: 1). 161 patients of the main group with a predisposition to VVLE and DVTL, which in turn were subdivided into a subgroup of patients: a) with varicose veins (VVLE) (n=111) and b) deep vein thrombosis of the lower extremity (DVTL) (n=50). As a control, 155 conditionally healthy individuals were examined without any predisposition to the onset and development of varicose veins and its complications. The main research method was PCR analysis. Isolation of genomic DNA was performed from peripheral blood lymphocytes of patients using the AmpliPrime RIBO-prep kit for isolation (Interlabservice LLC, Russia). The study was carried out by the method of quantitative real-time PCR analysis (Real-Time PCR). Amplification was performed using a thermal cycler for Real-Time PCR analysis - Rotor Gene Q, (Quagen,

Germany). For statistical processing of the results obtained, the application package "OpenEpi 2009, Version 9.3" was used.

Results and discussion. In the course of the study, distribution of the A and G alleles of this polymorphic locus was established among the patients of the main group, in the main and control groups. According to the data obtained, there were no statistically significant differences in the detection of alleles A and G. Thus, in the main and control groups, the A allele prevailed almost equally with a detection frequency of 92.5% and much less often the G allele was detected - in 7.4% of cases ($p = 0.9$; $RR = 1.0$; 95% CI: 0.75-1.34; $OR = 1.0$; 95% CI: 0.55-1.82).

The frequency of genotypes A/A, A/G, and G/G in the main group of patients and controls was 85.7%, 13.6% and 0.6% versus 85.8%, 13.5% and 0.6%, respectively (Fig. 1).

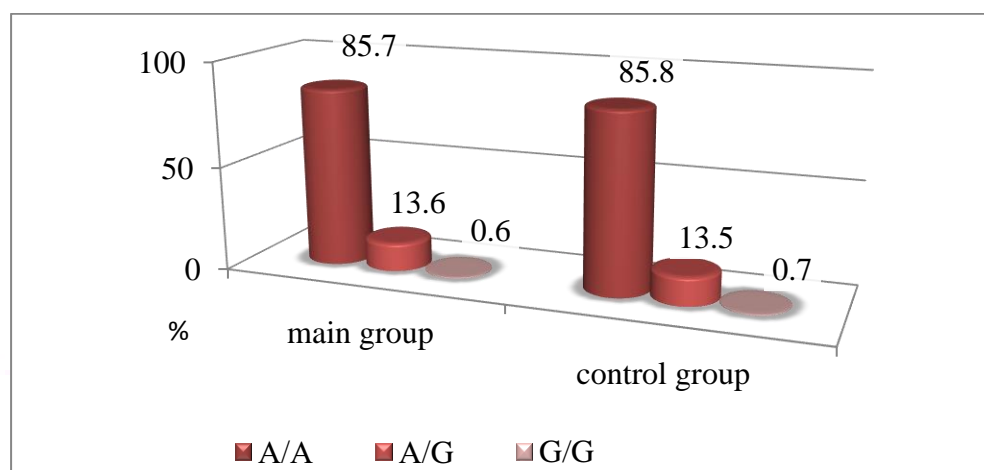


Figure 1. Frequency of distribution of rs2276109 polymorphism genotypes in the MMP12 gene in the study group of patients and in the control group

The A/A genotype was detected with almost the same frequency both in the main group and in the control groups (85.7% versus 85.8%, respectively ($p = 0.9$; $RR = 1.0$; 95% CI: 0.73-1.36; $OR = 1.0$; 95% CI: 0.53-1.87)). Heterozygous genotype A/G occurred with approximately the same frequency among patients and the control group: 13.6% versus 13.5%, respectively ($p = 0.9$; $RR = 1.0$; 95% CI: 0.73-1.38; $OR = 1.0$; 95% CI: 0.53- 1.92)). The frequency of the mutant homozygous genotype G/G was also the same in both groups: 0.6% versus 0.6%, respectively ($p = 0.9$; $RR = 1.0$; 95% CI: 0.24-3.95; $OR = 1.0$, 95% CI: 0.06-15.57)).

The results obtained in the course of the study showed statistically insignificant differences in the distribution of the A and G alleles of this polymorphism in the group of patients with varicose veins.

A allele in the group of patients with varicose veins and the control group was 91.9% and 92.5%, respectively ($\chi^2 = 0.1$; $p = 0.8$; $RR = 1.0$; 95% CI: 0.66-1.36; $OR = 0.9$; 95% CI: 0.48 -1.73)).

G allele in the group of patients with varicose veins and in the control group was detected with a frequency of 8.1% versus 7.4%, respectively ($\chi^2=0.1$; $p=0.8$; $RR=1.1$; 95%CI:0.74-1.52; $OR=1.1$; 95%CI:0.58-2.09)).

The frequency of genotypes A/A, A/G, G/G of the rs2276109 polymorphism in the MMP12 gene in the group of patients with varicose veins and controls was: 83.8%, 16.2% and 0.0% versus 85.8%, 13.5% and 0.6%, respectively. The frequency of detection of the wild A/A genotype was insignificantly higher in the control group: 83.8% versus 85.8%, respectively ($\chi^2=0.21$; $p=0.6$; $RR=0.9$; 95%CI:0.63-1.33; $OR=0.85$; 95%CI:0.43-1.68)). The frequency of detection of the heterozygous genotype A/G was higher among patients with varicose veins (16.2% versus 13.5%,

respectively $\chi^2=0.34$; $p=0.5$; $RR=1.1$; $95\%CI:0.77-1.63$; $OR=1.1$, $95\%CI:0.62-2.43$). The mutant homozygous genotype G/G was not detected in the group of patients with varicose veins (0.0% versus 0.6%, respectively, $\chi^2=0.7$; $p=0.4$).

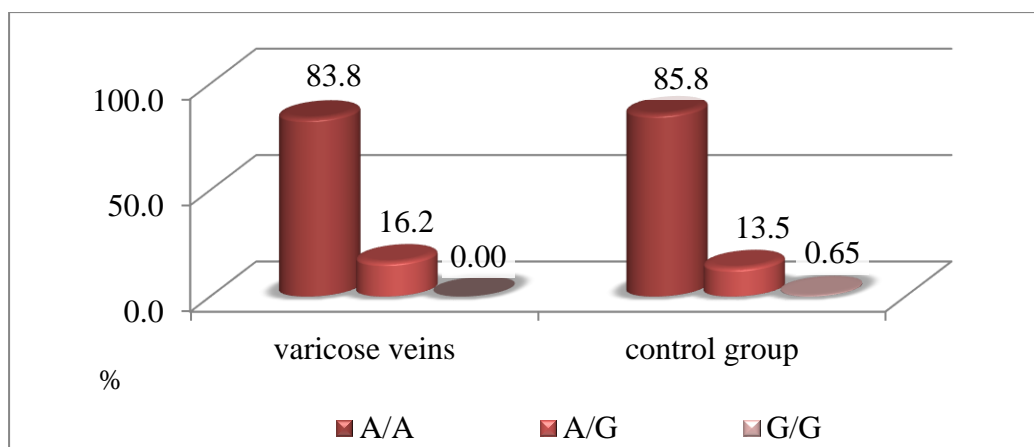


Figure 2. Frequency of distribution of rs2276109 polymorphism genotypes in the MMP12 gene in the group of patients with varicose veins and in the control group

The results of the study of the frequency of distribution of alleles and genotypes of the rs2276109 polymorphism of the MMP12 gene in the groups of patients with deep vein thrombosis of the lower extremities and in the control sample showed that the proportion of the A allele in the group of patients with venous thrombosis and the control group was 94.0% versus 92.5%, respectively ($\chi^2=0.2$; $p=0.6$; $RR=1.2$; $95\%CI:0.57-2.48$; $OR=1.3$; $95\%CI:0.50-3.18$).

G allele in the group of patients with venous thrombosis and the control group was 6% versus 7.4%, respectively ($\chi^2=0.2$; $p=0.6$; $RR=1.2$; $95\%CI:0.12-2.48$; $OR=1.3$; $95\%CI:0.50-3.18$).

Genotypes A/A, A/G, and G/G of the rs2276109 polymorphism in the MMP12 gene in the group of patients with varicose veins and controls were distributed as follows: 90.0%, 8.0%, and 2.0% versus 85.8%, 13.5% and 0.65%, respectively.

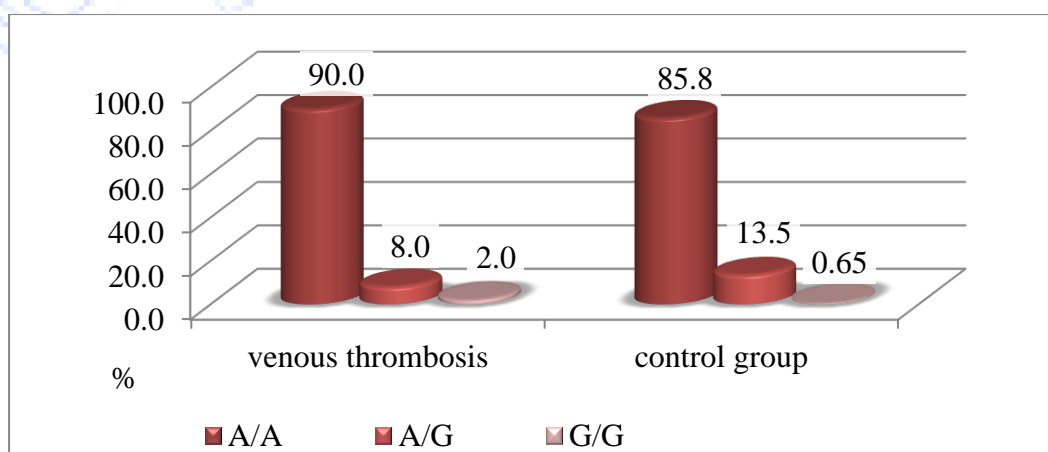


Figure 3. Frequency of distribution of rs2276109 polymorphism genotypes in the MMP12 gene in the group of patients with venous thrombosis and in the control group

The frequency of detection of the wild A/A genotype was insignificantly higher in the control group: 90.0% versus 85.8%, respectively ($\chi^2=0.6$; $p=0.4$; $RR=1.4$; $95\%CI:0.59-3.13$; $OR=1.5$; $95\%CI:0.33-4.16$). The frequency of detection of the heterozygous genotype A/G was statistically insignificantly higher in the control group compared to the sample of patients with varicose veins: 8.0% versus

13.5%, respectively ($\chi^2=1.0$; $p=0.3$; $RR=0.6$; 95%CI:0.25-1.61; $OR=0.6$; 95%CI:0.18-1.73). A mutant homozygous genotype G/G was detected in one patient with varicose veins complicated by venous thrombosis: 2.0% versus 0.6%, respectively ($\chi^2=0.6$; $p=0.4$; $RR=2.0$; 95%CI:0.48-8.09; $OR=3.0$, 95%CI:0.18-48.23).

According to the results obtained during the study of the frequency of distribution of alleles and genotypes of rs2276109 polymorphism in the MMP12 gene for the presence of differences in the group of patients with venous thrombosis, it was found that the carriage of the G/G genotype insignificantly increases the risk of phlebothrombosis by 3 times.

Discussion. To date, there are a lot of studies devoted to the study of the role of matrix metalloproteinases in the development of diseases of various organs and systems, however, in the structure of scientific works, there is very little material on the relationship between disturbances in the activity of the extracellular matrix metalloproteinase MMP-12, namely, a representative of zinc-dependent endopeptidases. One of the significant functions of MMP12 is elastin hydrolysis. Its hyperactivation and a decrease in the activity of its tissue inhibitors under conditions of varicose veins ultimately leads to the loss and rupture of elastin in the structure of collagen fibers and a decrease in the tone and dilatation of the venous walls [15].

According to the researchers, patients with relapse of varicose veins had a high incidence of the pathological gene MMP12 in 80.0%-both in homo- and heterozygous variants of the genotype, while in the first-time patients - only in a third of cases (33.3%) [16]. The authors established a statistically significant relationship between varicose veins and this gene (MMP12). Also, these authors have determined the relationship between the class of varicose veins and the frequency of detection of mutations in the MMP12 gene.

Differences in the expression of the MMP-12 protein in patients with varicose veins and in the norm were confirmed by foreign authors by methods of immunoblotting and immunohistochemistry [17].

According to Slonková V. et al, an association was established between the presence of the studied polymorphism of this gene in women with ulceration by 3.2 times, relative to women who did not have ulceration. The detection rate of the A/G genotype of the rs7123600 polymorphism of the MMP-12 gene was 4.7 times higher [18].

However, these data do not agree with the results of our study, according to which the carriage of an unfavorable genotypic variant of this polymorphism is not statistically significantly associated with an increased risk of developing VVLE and phlebothrombosis. This may be due to the peculiarities of the local population sample.

Output. The presence of an unfavorable allelic variant of the rs7123600 polymorphism of the MMP-12 gene cannot serve as an early marker of the development of structural changes in the vein wall and varicose veins of the lower extremities, as well as the development of deep vein thrombosis of the lower extremity. Despite the high $OR=3.0$, the statistical differences in the groups of patients with venous thrombosis and controls are statistically insignificant.

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