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Association of allellic polymorphism of tumor necrosis factor - alpha (rs1800629) factor with the development of immune microtrombovasculitis and immune trombicytopenia in adults

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Abstract

We studied the association of genetic rs1800629 TNFα polymorphism in the development of immune microthrombovasculitis (Shenlein-Henoch purpura) and immune thrombocytopenia in adults of Uzbek nationality. The rs1800629 polymorphism of the TNFα gene was evaluated by analyzing DNA samples obtained from peripheral blood by standard PCR. The results of the study showed the presence of a significant difference in the distribution of the allele A and the G / A genotype of the TNFα gene (rs1800629) among patients with immune microthromovasculitis (allele A: $\chi 2 = 4.89$; P = 0.027; OR = 2.53; 95% CI 1.09-5.89 and genotype G / A: $\chi 2 = 5.58$; P = 0.018; OR = 2.92; 95% CI 1.18-7.26) and immune thrombocytopenia (allele A: $\chi 2 = 5.05$; P = 0.024; OR = 2.44; 95% CI = 1 , 10-5.40 and genotype G / A: OR = 2.37; $\chi 2 = 3.86$; P = 0.049; 95% CI = 0.99-5.67) at the height of the disease relative to the control group, which in turn indicates the possible involvement of this polymorphism in the development of immune microthrombovasculitis and immune thrombocytopenia.

Keywords: rs1800629 polymorphism of the TNFα gene, immune microthrombovasculitis (Shenlein-Henoch purpura), immune thrombocytopenia, carriage, frequency, allele, genotype, association.

INTRODUCTION

Immune microtrombovasculitis (IMTV, Shenlein-Henoch purpura) and immune thrombocytopenia (ITP) are immune complex diseases with unclear etiology [2,5].

Studies aimed at studying the etiological and pathogenetic mechanisms of development of IMTV and ITP proved that diseases are associated with a variety of factors, which allows them to be defined as multifactorial [1, 3].

The molecular basis underlying the development of IMTV and ITP is not fully understood; however, some facts confirm the opinion that genes play a crucial role in the pathogenesis of these diseases [6,11,17,19].

It is known that cytokine genes play an important role in the implementation of immune and inflammatory processes in the human body, polymorphic changes in which lead to disturbances in the regulation of inflammation and the immune response [9,10]. Of a large number of families of all cytokine genes, one of the most important is the pro-inflammatory cytokine gene TNF-α produced mainly by activated macrophages [21].

It is assumed that TNF-α increases during the acute stage of IMTV and may enhance the binding activity of IgA antibodies against endothelial cells, and therefore the potential role of TNFα gene polymorphism (rs1800629; G 308A) in the pathogenesis of IMTV was evaluated in a number of studies [7,20,23]. However, some authors cite the absence of a link between the genetic polymorphism

of TNF-α (rs1800629) and the development of the disease [20], while others, on the contrary, claim its presence [7].

The role of this cytokine gene is also evaluated in the development of ITP [17,18]. Thus, Turkish researchers T. Sever, S. Oguzkan, T. Babacan (2011) found high expression of the TNFα gene (-308) (OR = 0.249, 95% CI: 0.076-0.815, p <0.05) in patients with the chronic form ITP (OR = 0.318; 95% CI: 0.103-0.987) [18].

At the same time, E. Okulu, T. İleri, V. K. Çulha, et al. (2011) after studying the role of the role of tumor necrosis factor-alpha (TNF-α) -308 G / A in the development and clinical progression of ITP in 50 patients, it was concluded that the risk of ITP development and clinical progression are not associated with TNFα gene polymorphism (G308A) (OR = 0.738; 95% CI: 0.275-1.981 and OR = 0.762, 95% CI: 0.179-3.249) [13].

Thus, the existing data on the study of the association of TNF-a with the formation of IMTV and ITP are contradictory. In connection with the presence of conflicting opinions in this regard, studying the association of the rs 1800629 polymorphism of the TNF-α gene with the risk of developing IMTV and ITP in people of Uzbek nationality is interesting.

MATERIAL AND METHODS

The study included 169 unrelated persons of Uzbek nationality, among whom 75 (1st group) included patients diagnosed with immune microthrombuscitis established based on modern classification criteria EULAR, PRINTO and PreS (2010) [14] and 89 (2nd group) - patients with immune thrombocytopenia, verified on the basis of recommendations of international experts (2009) [16]. All patients (age range from 16 to 80 years) were observed in the consultative and diagnostic clinic of the Research Institute of Hematology and Blood Transfusion of the Ministry of Health and Social Development in the period from 2017 to 2018. Each group, depending on the stage of the disease, is subdivided into two subgroups: “A” subgroup - high phase and “B” subgroup - remission stage of the disease. The control group consisted of conditionally healthy non related persons of Uzbek nationality, corresponding to the sex and age of the examined groups of patients.

DNA was isolated from venous blood leukocytes in accordance with the standard DNA isolation protocol [12]. Detection of the rs1800629 polymorphism of the TNFα gene was performed using SNP-PCR on a programmable thermal cycler from Applied Biosystems 2720 (USA), using test systems from Litex (Russia), according to the manufacturer's instructions.

Statistical analysis of the results was carried out using the statistical software package "OpenEpi 2009, Version 9.3".

RESULTS AND DISCUSSION

We have investigated the biallelic polymorphism rs1800629 of the TNF-α gene, which is the replacement of a single guanine nucleotide by adenine (G / A). In our observations, in examined patients with IMTV (n = 75) compared with the control group, the incidence of unfavorable allele A increased 1.7 times - from 7.3% (in control) to 12.7% (in the main group) with variations from 10.3% in patients “B” of a subgroup and up to 14.6% in patients of “A” a subgroup (Table 1.).

Table 1: Analysis of the results of the study of polymorphism rs1800629 TNFα gene in groups of patients with IMTV and control.

Group	n	Allele Frequency				Genotype distribution Frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	N	%	n	%	n	%
The main group of IMTV	75	131	87.3	19	12.7	56	74.7	19	25.3	0	0
"A" subgroup	41	70	85.4	12	14.6	29	70.7	12	29.3	0	0
"B" subgroup	34	61	89.7	7	10.3	27	79.4	7	20,6	0	0
Control group	73	135	92.3	11	7.3	62	84.9	11	15.1	-	0

A significant increase in the carrier frequency of the adverse A allele in the main group of patients with IMTV ($\chi 2 = 3.21$; P = 0.073; OR = 2.0; 95% CI 0.93-4.31), and in subgroups of patients "A" - up to 14.6% ($\chi 2 = 4.89$; P = 0.027; OR = 2.53; 95% CI 1.09-5.89) and "B" - up to 10.3% ($\chi 2 = 0.46$; P = 0.50; OR = 1.41; 95% CI 0.52- 3.81) indicates the association of this allele with an increased risk of developing IMTV in the “A” subgroup (Table 2).

Table 2: An associative connection between the rs 1800629 polymorphism of the TNF-α gene and the risk of developing IMTV in comparison with the control

Polymorphism		Allele, genotypes	Control group, (n = 73)		Maingroup, (n = 75)		Credibility
			n	%	n	%	
308 G/A TNF-α	Allele	308G	135	92,5	129	86,0	χ^2=3.208; p=0.07329; OR=1.998; 95% CI: 0.9266-4.308)
		308A	11	7,5	21	14,0	
	Genotypes	308 G/G	62	84,9	54	72,0	χ^2=3.65; p=0.05606; OR=2.192; 95% CI: 0.9697-4.955)
		308 G/A	11	15,1	21	28,0	
		308 A/A	0	0	0	0	

It should be noted that in control sample and in patients with IMTV, only wild G / G and heterozygous G / A genotypes were found, and the carriage of unfavorable rare genotype A / A was not detected. Along with this, in the group of patients, in comparison with the control, the heterozygous G / A genotype was registered 1.7 times more often (25.3 % versus 15.1%), while the highest percentage of carriers of this genotype was noted in the “A” subgroup of patients (29.3%).

The carrier frequency distribution of the heterozygous genotype G / A in the main group of patients with IMTV (25.3% versus 15.1; χ2 = 3.65; P = 0.056; OR = 2.19; 95% CI 0.97-4.96), and in subgroups of patients "A" (29.3 % versus 15.1; χ2 = 5.58; P = 0.018; OR = 2.92; 95% CI 1.18-7.26) and B (20.6% versus 15.1%; χ2 = 0.51; P = 0.48; OR = 1.46; 95% CI 0.51 - 4.18) indicates the association of this allele with an increased risk of developing IMTV in the “A” subgroup.

The above frequency distribution of the genotypes of the rs1800629 polymorphism of the TNFα gene (Hobs) both in the main group of patients and in the control group corresponded to the expected distribution (Hexp) according to the Hardy-Weinberg equilibrium.

Frequency analysis of the distribution of alleles in the ITP patient group (n = 89) showed a decrease in the frequency of the G allele (83.7% versus 92.3%) in comparison with that in the control group.

At the same time, the examined patients showed a significant increase in the incidence of unfavorable allele A from 7.4% (in the control group) to 16.3%. At the same time, the same peculiarity was also detected in the subgroups of ITP patients: in the “A” subgroup (n = 49) the share of the G and A allele was 83.7% and 16.3%; in the “B” subgroup (n = 40), their values were 83.8% and 16.2%.

In the main group of patients, the frequency of the homozygous genotype G / G was detected in 68.5% (n = 61), and that of the heterozygous genotype G / A in 30.3% (n = 27) cases, whereas in the control group the frequency of genotype G / G turned out to be more (85.2%), and G / A genotype less (14.8%). At the same time, it should be noted that along with the indicated genotypes in one case in the main group of patients, due to subgroup “A”, the presence of mutant homozygous genotype A / A (1.1%) was recorded, while in the control group the carriage of this genotype was not observed (Table 3).

Table 3: The distribution frequency of alleles and genotypes of gene polymorphism TNFα (rs1800629) in the control group and in patients with ITP

Group	n	Allele Frequency				Genotype distribution Frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
The main group, of them:	89	149	83,7	29	16,3	61	68,5	27	30,3	1	1,1
"A" is a subgroup	49	82	83,7	16	16,3	34	69,4	14	28,6	1	2,0
"B" - a subgroup	40	67	83,8	13	16,2	27	67,5	13	32,5	0	0
Control group	81	150	92,3	12	7,4	69	85,2	12	14,8	0	0

Studying the differences in the frequency distribution of alleles and genotypes of the TNFα gene polymorphism (rs1800629) show that the main group of patients compared to the control group allele G was significantly lower by 1.1 times, while the frequency of allele A increased almost twice (χ2 = 3.208; p = 0.07329; OR = 1.998; 95% CI: 0.9266 4.308), with respect to the genotypes, the frequency of the G / G genotype was higher (85.2%), and the G / A genotype was less (14.8%) (χ2 = 3.65; p = 0.05606; OR = 2.192; 95% CI: 0.9697-4.955) (Table 4).

Table 4: Differences in the frequency distribution of alleles and genotypes of the TNFα gene polymorphism (rs1800629) in the control group and in ITP patients

Polymorphism		Allele, genotypes	Control group, (n = 81)		Main group, (n = 89)		Reliability
			n	%	n	%	
308 G/A TNF-α	Allele	308G	150	92,3	149	83,7	χ^2=6,31; p=0.0012; OR=1.998; 95% CI: 0.9266-4.308)
		308A	12	7,4	29	16,3	
	Genotypes	308 G/G	69	85,2	61	68,6	χ^2=3.65; p=0.05606; OR=2.192; 95% CI: 0.9697-4.955)
		308 G/A	12	14,8	27	30,3	
		308 A/A	0	0	1	1,1	

In the “A” subgroup of ITP patients who were at the height of the disease, the proportion of the carrier of the favorable G allele more than once was lower, the frequency of the unfavorable A allele was 2.44 times statistically significantly higher than those in the control group (χ2 = 5.05 ; p = 0.024; OR = 2.44; 95% CI: 1.10-5.40). At the same time, compared with the control group, the homozygous G / G genotype was significantly lower by 1.23 times, the heterosygous G / A genotype significantly exceeded 2.9 times (χ2 = 5.581, p = 0.01815, OR = 2.923; 95% CI: 1.177-7.259). It is important to note that, although in a single case, it was only in this subgroup of patients that the mutant genotype A / A was detected (2.0%).

Studying the difference in the “B” subgroup of ITP patients who were in remission shows that the proportion of the carrier of the unfavorable allele A was 2.5 times statistically significantly higher than the share in the control group (χ2 = 4.894; p = 0.02695; OR = 2.527; 95% CI : 1.089-5.863), while the proportion of homozygous G / G genotype was significantly lower, and the heterosygous genotype G / A statistically significantly exceeded those in the control (χ2 = 5.581; p = 0.01815; OR = 2.923; 95% CI: 1.177-2.759).

The above frequency distribution of the genotypes of the rs1800629 polymorphism of the TNFα gene (Hobs) both in the main group of patients with ITP and in the control group corresponded to the expected distribution (Hexp) according to Hardy-Weinberg law.

This fact suggests that the heterozygous G / A genotype of the rs1800629 polymorphism of the TNF-α gene is reliably associated with the development of immune microthrombovasculitis and immune thrombocytopenia in people of Uzbek nationality. This is probably due to the loss of the protective effect of the wild G / G genotype in individuals with a heterozygous type of TNF-α gene polymorphism (rs1800629). These results contribute to the formation of fundamental ideas about the molecular genetic basis and pathogenetic mechanisms of development of these diseases in people of Uzbek nationality.

CONCLUSION

Immune microtrombovasculitis and immune thrombocytopenia are diseases, the etiopathogenetic mechanisms of which have not yet been fully studied [2,5]. Today there are a number of opinions and statements that genetic polymorphisms determine decisive role in the pathogenesis of these diseases [1,4]. To date, many molecular genetic studies have been carried out to study the role of genes in the formation of IMTI and ITP. However, data from studies conducted in different populations are controversial, possibly due to ethnic differences with a predisposition

to the development of this disease. The literature describes the results of studies on the role of the tumor necrosis factor-α gene (TNF-α), a proinflammatory cytokine that is involved in systemic inflammation and in the pathogenesis of inflammatory diseases [15, 23]. According to Ding GX et al. (2016) [7], the G308A allele A of the TNF-α gene increases the risk of developing immune microthrombovasculitis, while other authors did not find an association of the TNF-α gene with the development of the disease [20]. Also, controversial data were obtained when studying the contribution of this genetic marker and in the development of ITP [8,21,22]. So, Yadav D. K., Tripathi A. K. et al. (2016) studying the features of the tumor necrosis factor-TNF-α gene polymorphism in Indian patients with ITP did not reveal significant differences in the distribution of the genotype of the heterozygous version of the TNF-α gene among patients and controls [22]. While the results of the research El Sissy A.H., Elanwary Sh. (2014) showed a significant increase in the frequency of the homozygous genotype A / A 6 times and almost 2 times the heterozygous genotype G / A of the TNFα gene polymorphism (G308A) in patients with ITP in relation to those in the control [8].

This study examined the genetic association of the TNF-α gene polymorphism (rs1800629) with the development of IMTI and ITP. The results of our studies showed that in the mid-stage, the frequency of occurrence of an unfavorable allele A (14.6% versus 7.3%) and the heterozygous G / A genotype (29.3% versus 15.1%) of the TNFα gene (rs1800629) in the group of patients with immune microthrombovasculitis, as well as the frequency of allele A (16.3% versus 7.4%) and the heterozygous G / A genotype (28.6% versus 14.8%) of the TNFα gene (rs1800629) in the group of patients with immune thrombocytopenia is significantly higher compared to the control group, which in turn indicates about the possible involvement of this genetic polymorphism ma in the pathogenesis of these diseases.

Analyzing the results of existing studies on the study of molecular genetic mechanisms of development of IMTI and ITP, given their controversial nature, it is obvious that the genetic mechanism of the development of the disease is very complex and not fully understood.

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