THE RESEARCH OF rs1800629 POLYMORPHISM OF TNF-a GENE IN BLOOD OF DIABETIC RETINOPATHY PATIENTS

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ABSTRACT:

Diabetic retinopathy (DR) is a serious complication of diabetes and the main cause of visual impairment and blindness in persons of working age. Cytokines play a key role in the immunopathogenesis of autoimmune DM. Among them is tumour necrosis factor-α (TNF-α). The aim of this study was to study the polymorphism of rs1800629 gene TNFα in patients with type 2 DM. The study included 40 patients with type 2 DM (17 men and 23 women) aged 48 to 70 years (mean age - 51±2.92 years) without retinopathy, as well as with NDR, and 50 practically healthy persons (mean age - 31.2±0.5 years) undergoing ophthalmological examination in RSSPMCEM. The initial stage of our work was modification of TNF-G-308A polymorphism testing method (rs1800629). To perform molecular genetic studies of rs1800629 polymorphism of TNF- gene we designed systems of oligoprimers based on polymerase chain reaction in real time. Thus, the study of the rs1800629 polymorphic DNA locus of the TNF-α gene did not reveal reliable differences in the distribution of allele and genotype frequencies between the main sample and the population sample. However, there was a tendency to increase the frequency of functionally unfavourable allele A and heterozygous genotype G/A among patients in comparison with control, which requires confirmation in a larger sample of patients.

Keywords: non-proliferative diabetic retinopathy, polymorphism of rs1800629 gene TNF-a.

INTRODUCTION:

Diabetic retinopathy (DR) is a serious complication of diabetes and the main cause of visual impairment and blindness in persons of working age [1]. Chronic hyperglycemia is known to be the main etiological factor leading to all microvascular complications of diabetes, including diabetic retinopathy. Currently, there is an active search for genes responsible for the formation of predisposition to diabetic

retinopathy. Numerous molecular genetic studies of type 2 DM and few results of genetic studies of DR, combining the capabilities of candidate and position mapping methods, open up new data on the genetic basis and peculiarities of pathophysiology of this disease. Nevertheless, despite the successes of genetics, molecular biology and immunology, there are still many unresolved issues. A comprehensive study of diabetic retinopathy in various ethnic groups in type 2 DM and the study of the genetic basis of the pathology may allow the future development of new approaches to prevent and treat the disease. Cytokines [2.3.4..5] play a key role in immunopathogenesis of autoimmune DM. They include tumour necrosis factor-α (TNF-α). Under physiological conditions, TNF-α manifests itself as an immunoregulator, participates in the processes of proliferation and differentiation of various cell types, influences cell apoptosis and stimulates the production of various cytokines [6,7,8]. Tumor necrosis factor (TNFa) is a multifunctional cytokine participating in all inflammatory reactions and being a key participant of pathogenesis of inflammatory, autoimmune and malignant diseases [9,10].

Researches of associations of polymorphic loci of TNFa gene with risk of diabetes mellitus development and its complications were carried out in different populations, as a result of which contradictory results were obtained. The association of rsl800629 (-308G/A) polymorphous loci with the development of type 2 DM and insulin resistance in European populations was found; at the same time, no significant associations of rsl800629 polymorphous variant with diabetes[11,12,13,14,15] were found by meta-analysis results. The increased risk of development of macrovascular complications in Caucasians from Canada, patients with type 2 DM, was established in carriers of TNFa allele -308A[16].

The association of rsl799724(-853C/T) polymorphic locus of TNFa gene with the risk of diabetes development in overweight Japanese is established. In haplotype analysis of 6 polymorphic variants (rs2229094A/G, rsl041981 C/A, rsl800630C/A,rsl800629G/A, rs361525G/A, rsl80061 OS/T) of TNF-LTA chromosomal section in India, patients with type 2 SD, increased frequency of ACCGGC haplotype was found in comparison with the control sample. According to the literature, increased expression of genes of proinflammatory cytokines, such as interleukin 1 and TNFa, was detected in the retina[17]. In addition, in experiments using animal models it was found that TNFa inhibition has a positive effect in diabetic retinopathy [18,19]...

The obtained results testify to the undoubted effect of TNFa on the development of DR, type 2 DM and its other complications[21].

The aim of this research was to study rs1800629 polymorphism of TNFa gene in patients with type 2 DM.

Material and methods.

The study included 40 patients with type 2 DM (17 men and 23 women) aged 48 to 70 years (mean age - 51±2.92 years) without retinopathy, as well as with NDR, and 50 practically healthy persons (mean age - 31.2±0.5 years) who underwent ophthalmological examination in RSSPMCEM. All patients with type 2 DM were divided into 2 groups: Group 1 - 22 patients with type 2 DM without signs of DR (9 men and 13 women) aged 48 to 65 years (mean age - 55±4.63 years); group 2 - 18 patients with MD (8 men and 10 women) aged 49 to 70 years (mean age - 57.56±2.27 years).

The diagnosis of type 2 diabetes was made by an endocrinologist in accordance with ICD criteria. The diagnosis of NDR was made on the basis of ophthalmoscopy data with the maximum dilated pupil. The stage of retinopathy was established according to the WHO classification according to which diabetic non-proliferative retinopathy is characterized by the following retinal changes: the presence of pathological changes in the retina in the form of microanneurysm, dot or small darkened hemorrhages localized in the central zone of the eye bottom or along large veins in deep layers of the retina, exudative foci (localized in the central part of the eye bottom, yellow or white with clear or vague borders) and retinal edema, localized in the central (macular) region or in the course of large vessels.

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PCR genotyping of rs1800629 polymorphism of TNF- gene rs1800629 was performed in the laboratory of molecular genetics of the Research Institute of Hematology and Blood Transfusion in all patients with DM and in 50 healthy unrelated Uzbek donors who formed the control group. The blood was taken on an empty stomach from the ulnar vein of the examined patients under sterile conditions.

The initial stage of our work was a modification of TNF-G-308A polymorphism testing method (rs1800629). To perform molecular genetic studies of rs1800629 polymorphism of TNF- gene we designed a system of oligoprimers based on polymerase chain reaction in real time. The research was conducted in accordance with the test set developed by us together with the staff of the Research Institute of Hematology and Transfusiology (St. Petersburg) (Head of Laboratory, Dr. Kapustin S.I.). The proposed test system is based on real-time PCR analysis of rs1800629 polymorphism of TNF-gene using 2 fluorescent probes in one tube.

Oligo v.6.31 (Molecular Biology Insights Inc., USA) has selected the oligonucleotide primers required to perform PCR of these fragments. The characterization of the sequence of synthesized oligoprimers is given below:

F: 5'-AATAGGTTTTGAGGGCCATG-3'
R: 5'-ATCTGGAGGAAGCGGTAGTG-3'

The collection of other reagents, enzymes and other components, including reagents for the extraction of nucleic acids, were purchased from the world's leading manufacturers (Serva, Sigma, etc.) and Russia (Sintol, NPF Litech, DNA technology, Sibenzim, etc.) working in the field of molecular biology.

It is known that testing of a particular polymorphism or gene mutation often requires precise information about the number and purity of a sample of isolated DNA. DNA isolation from peripheral blood lymphocytes was performed using the RNA-Sorb set (Interlabservis, Moscow). In addition, we modified the method of phenolic chloroform extraction of DNA. The concentration and purity of extracted DNA was measured on the NanoDrop 2000 spectrophotometer (USA) at A260/280 nm wavelength. The purity of the extracted DNA samples, determined by the A260/280 ratio, was 1.7/1.8. This indicates very low levels of contaminants or other macromolecules in the isolated DNA solutions and these samples can be used in real-time PCR without further purification.

The estimation of deviation of frequencies of observed and expected genotypes from the canonical distribution of Hardy-Weinberg was carried out with the help of computer program "GenePop". The deviation coefficient was calculated using a formula: D=(Hobs-Hexp)/Hexp, where: hobs and hexp are the observed and expected heterozygosity, respectively. The "OpenEpi 2009, Version 2.3" application package was used as a statistical calculation tool.

The results: The duration of DM current in patients was 7.5±1.8 years on the average. At the analysis of clinical and functional parameters of the patients with DM it was revealed that in 22 patients with DM of type 2 there were no signs of DR and average visual acuity with correction made 0.7±0.12. In patients with PDD (18 patients) at ophthalmoscopy in the conditions of medication mydriasis on the eye bottom microanneurysms, petechial hemorrhages, capillary dilation, in some places solid exudates were found out. The average visual acuity with correction in these patients was 0.42±0.11. All patients had tonometric IOP within the normal range: from 16 to 22 mm Hg. (on the average 18.2±2.9 mm Hg).

It was found that the frequency distribution of genotypes and alleles of rs1800629 of the TNF-gene in both groups corresponded to the expected Hardy-Weinberg equilibrium law ($p>0.05$) (Tables 1-3).

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Table 1

Expected and observed frequencies of genotype distribution by PCV in the group of DR patients

Genotypes	**Genotype Frequency**		χ^2	
	Observed by H_{obs}	***Expected.*** H_{exp}		
G/G	0.8	0.81	,005	
G/A	0.2	0.18	,089	
A/A	0,00	0.01	,400	
TOTAL	1,00	1,00	,494	**.5**

Table 2

Expected and observed frequencies of distribution of genotypes by PCB in the population sample.

Genotypes	**Genotype Frequency**		χ^2	
	Observed by H_{obs}	***Expected.*** H_{exp}		
G/G	0.88	0.88	,001	
G/A	0.12	0.11	,023	
A/A	0,00	0.04	,180	**.6**
Всего	1,00	1,00	,204	

The evaluation of differences between observed and expected heterozygote frequencies in the studied group of patients and controls revealed that this polymorphism has a very low heterozygote deficit index (from 0.2/0.18 and 0.12 / 0.11, respectively). Meanwhile, the relative deviation of the expected heterozygosity from the one observed in both groups was positive, i.e. the index D was >0 (D =+0.11 and D =+0.09). (Table 3).

Table 3

Relative deviation of expected heterozygosity from observed (D)

Groups	H_{obs}	H_{exp}	**D***
Main group	0.2	0.18	+0.11
Controlgroup	0.12	0.11	+

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			0.09

Note: D=(0.2-0.18)/0.18 =+0.11 for the main group;
D=(0.12-0.11)/0.11=+0.09 for the control group.

Comparative analysis of the allele frequencies and genotypes of rs1800629 polymorphism of the TNF- gene between the group and the population group revealed statistically insignificant differences. The allele G and A allele frequency in the main group and control group were 90.0% and 10.0% and 94.0% and 6.0%, respectively (Table 4).

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Table 4.

Distribution frequency of alleles and genotypes of polymorphism rs1800629 of TNF-α gene in patient groups and controls

Group	Alele Frequency				Frequency of genotypes distribution					
	G		A		G/G		G/A		A/A	
					n	%				
Maingroup (n=40)	2	0,0		0,0	2	0,0		0,0		
Controlgroup (n=50)	4	4.0		.0	4	8.0		2.0		

χ^2=1.0; P=0.3; OR=1.7; 95% CI 0.5783, 5.24

χ^2=1.0; P=0.3; OR=0.5; 95% CI 0.1723, 1.726

χ^2=1.0; P=0.3; OR=1.8; 95% CI 0.5792, 5.803

At the same time, the heterozygous variant G/A rs1800629 of the TNFα gene was found to be frequently present in 2.0% of patients compared to 12.0% control (χ2=1.0; P=0.3; OR=1.8; 95% CI 0.5792, 5.803), indicating a potential adverse role of this genotype in the development of diabetic retinopathy.

Conclusion.

Thus, the study of the polymorphic DNA locus rs1800629 of the TNF-α gene did not reveal reliable differences in the distribution of alleles and genotype frequencies between the main sample and the population sample. However, there was a tendency to increase the frequency of functionally unfavorable allele A and heterozygous genotype G/A among patients in comparison with control, which requires confirmation in a larger sample of patients.

Reference:

1. Centers for Disease Control and Prevention: National diabetes statistics report: estimates of diabetes and its burden in the United States, 2014. Atlanta, GA, CDC, 2014. Available at

2. Kravets E.B., Saprina T.V., Lazarenko F.E. and others. The role of cytokines in pathogenesis of autoimmune diabetes, questions of immunointervention (in Russian) // Bul.sib.med. 2010. T.9, No1. pp.76—83

3. Saprina T.V., Lazarenko F.E., Prokhorenko T.S. and others. Features of Basal and Stimulated Interleukin-2 and -4 Blood Mononuclear Secretion in Auto-immune Diabetes // Sib.med. zhurnal. 2010. T.25, No1. pp.41—44.

4. Ban L., Zhang J., Wang L.Selective death of autoreactive T-cells in human diabetes by TNF or TNF receptor 2 agonism //The National Acad. of Scien. 2008. V.105, No36. P.13644—13649

5. Rabinovitch A., Suarez-Pinzon W.L.Roles of cytokines in the pathogenesis and therapy of type 1 diabetes // Cell Bio-chem Biophys.2007. V. 482, No3. pp.159—163

6. Schneider-Brachert W., Tchikov V., Neumeyer J. et al.Com-partmentalization of TNF receptor 1 signaling; internalized TNF receptosomes as death vesicles // J. Immunity. 2004. V. 21, No3. pp. 415—42

7. Schütze S., Tchikov V., Schneider-BrachertW.Regulation of TNFR1 and CD95 signalling by receptor compartmentalization // Nat.Rev.Mol.Cell.Biol. 2008. V. 9, No8. P.655—662

8. Uno S., Imagawa A., Okita K. et al.Macrophages and den-dritic cells infiltrating islets with or without betacells produce tumour necrosis factor-alpha in patients with recentonset type 1 diabetes // Diabetologia. 2007. V. 50, No3. P.596—60

9. A novel serum protein similar to Clq, produced exclusively in adipocytes / P. E.Scherer, S. Williams, M. Fogliano [et al.] // J. Biol. Chem. - 1995. - Vol. 270. - P. 26746 -26749.

10. The human apM-1, an adipocyte-specific gene linked to the family of TNF's and to genes expressed in activated T cells, is mapped to chromosome Iq21.3-q23, a susceptibility locus identified for familial combined hyperlipidaemia (FCH) / A. Schaffler, E. Orso, K.D.Palitzsch [et al.] // Biochem. Biophys. Res. Commun. - 1999. - Vol. 260. - P. 416-425.

11. Association of the tumour necrosis factor a-308G/A polymorphism with the risk of diabetes in an elderly population-based cohort / B. T. Heijmans, R. G. Westendorp, S.Droog [et al.] // Genes Immun. - 2002. - Vol. 3. - P. 225-228.

12. Paine, S. K. Association of tumor necrosis factor alpha, interleukin 6, and interleukin 10 promoter polymorphism with proliferative diabetic retinopathy in type 2 diabetic subjects \S. K. Paine, A. Sen, S. Choudhuri // Retina. - 2012. - Vol. 32, № 6. - P. 1197-1203.

13. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: The Finnish Diabetes Prevention Study / A. Kubaszek, J. Pihlajamaki, V. Komarovski [et al.] // Diabetes. - 2003.Vol. 52.-P. 1872-1876.

14. Sookoian, S. C. Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome /S. C. Sookoian, C Gonzalez,C.J.Pirola // Obes. Res. - 2005. - Vol. 13. - P. 2122-2131.

15. Tumor necrosis factor alpha -238G >A genotype alters postprandial plasma levels of free fatty acids in obese individuals with type 2 diabetes mellitus. / B. Fontaine-Bisson, T.M. Wolever, J. L. Chiasson [et al.] // Metabolism. - 2007. - Vol. 56. - P. 649-655.

16. Association between LTA, TNF and AGER polymorphisms and late diabetic complications / E. Lindholm, E. Bakhtadze, C Cilio [et al.] // PLoS ONE. - 2008. - Vol. 3. P. e2546.

17. Systemic soluble tumor necrosis factor receptors 1 and 2 are associated with severity of diabetic retinopathy in Hispanics / J. Z. Kuo, X. Guo, R. Klein [et al.] // Ophthalmology. - 2012.-Vol. 119, № 5 . - P . 1041-1046.

18. A central role for inflammation in the pathogenesis of diabetic retinopathy / A.M.Joussen, V. Poulaki, M. L. Le [et al.] // Faseb. J. - 2004. - Vol. 18. - P. 1450-1452.

19. Suppression of diabetic retinopathy with angiopoietin-1 / A. M. Joussen, V. Poulaki, A. Tsujikawa [et al.] // Am. J. Pathol. - 2002. - Vol. 160. - P. 1683-1693

20. Association of the tumour necrosis factor a-308G/A polymorphism with the risk ofdiabetes in an elderly population-based cohort / B. T. Heijmans, R. G. Westendorp, S.Droog [et al.] // Genes Immun. - 2002. - Vol. 3. - P. 225-228.

21. The adipocyte-secreted protein Acrp3O enhances hepatic insulin action / A. H. Berg,T.P. Combs, X. Du [et al.] // Nat. Med. - 2001. - Vol.17. - P. 947-953.

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