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TNF- α Gene G308A Polymorphism: Frequency in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Tumour necrosis factor-alpha (TNF- α), a pro-inflammation cytokine, plays a critical role in the pathogenesis of type 2 diabetes mellitus due to involving into the regulation of insulin signalling. Overproduction of this cytokine in metabolic syndrome is linked to TNF- α promoter gene G-308A polymorphism.

Objective: The work was initiated to measure the serum TNF- α concentrations and to determine the frequency of the Tumour necrosis factor-alpha (TNF- α) gene G308A polymorphism in Uzbek patients with type 2 diabetes mellitus.

Methods: Healthy and type 2 diabetes mellitus Uzbek patients were observed.

Results: In the group with diabetes fasting blood glucose and the glycated haemoglobin (HbA1c) levels were 2.7 and 1.6 times higher respectively (both $p < 0.01$). HDL cholesterol was reduced, while LDL cholesterol, total cholesterol and triglycerides were higher in diabetic group. There was a significant difference between the serum TNF- α concentration in people with and without DM. As to TNF- α gene G308A polymorphism in the sample under study, it was represented mostly by GG homozygous genotype to be registered among persons without diabetes (90.2%) and patients with type 2 DM (83.3%). GA heterozygous genotype occurred in 9.8% and 16.7% of non-diabetics and diabetics, respectively. In our study, pathological AA homozygous genotype was found neither among the diabetics nor in the controls. There were no significant differences in frequencies of alleles and genotypes of TNF α gene G308A polymorphism. In the diabetics, frequencies of A allele and GA genotype is insignificantly higher than those in the controls.

Conclusion: In compare to non-diabetic controls, patients with type 2 diabetes mellitus had higher serum concentrations of TNF- α . However, in that small group association of A allele and GA genotype of TNF- α gene with a higher risk of DM2 were insignificant.

Key Words: Type 2 diabetes mellitus, Cytokines, Tumour necrosis factor-alpha, TNF- α gene polymorphism

INTRODUCTION

Over the last few years, roles of cytokines in the pathogenesis of human diseases are being studied intensively.^{1,2} The Tumour necrosis factor-alpha (TNF- α), a pro-inflammatory cytokine, is a highly active polyfunctional cytokine playing a key role in the pathogenesis of diseases such as Rheumatoid arthritis, Parkinson Disease, Obesity, Diabetes Mellitus (DM) and making an impact on the regulation of metabolism in a human organism.³

Under physiological conditions, TNF- α plays a critical role in the regulation of normal differentiation, growth and metabolism of various cells involved in mechanisms of cell death and apoptosis. On the other hand, TNF- α acts as a mediator of inflammation in various human diseases.^{1,2} Findings from multiple studies demonstrated interrelation between increase in TNF- α levels and the initiation of inflammation and cancer, as well as of some metabolic, autoimmune and neurodegenerative diseases.^{2,5-7} There is convincing evidence of TNF- α as a key pro-inflammatory

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mediator involved in insulin resistance and type 2 diabetes mellitus pathogenesis. It is a trigger in obesity, diabetes mellitus and diabetic complications^{1,3}

Intense production of TNF- α was found to correlate with G-308A polymorphism in the promoter region of TNF- α gene.⁸ TNF- α gene G308A polymorphism was demonstrated to govern the onset of hyperinsulinemia, arterial hypertension and obesity.⁹⁻¹¹ Studies on TNF- α and its gene polymorphisms may be of predictive value in the estimation of risk of type 2 diabetes mellitus and its complications.

Our investigation aimed to determine the frequency of the Tumour necrosis factor-alpha (TNF- α) gene G308A polymorphism in patients of Uzbek nationality with type 2 diabetes mellitus.

MATERIALS AND METHODS

We examined 48 Uzbek patients with type 2 diabetes mellitus of mean age 60.6 ± 7.3 years, BMI $< 30 \text{ kg/m}^2$ and mean disease duration 7.67 ± 5.4 years. Forty-one people of Uzbek nationality, in average age 57.4 ± 8.6 without clinical and laboratory signs of carbohydrate metabolism disturbances were included in the control group. All participants were examined at the Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Ministry of Health of the Republic of Uzbekistan. The glucose oxidase test was used to measure fasting capillary blood glucose employing kits from the "Cypress Diagnostics" (Belgium). An automatic analyzer DCA Vantage (Siemens, Germany) was used to measure HbA1c. Triglycerides, total cholesterol, as well as HDL cholesterol and LDL cholesterol were measured employing reagents provided by the Human GmbH (Germany) on a biochemical analyzer BA-88A (Mindray Medical International Ltd, China). ELISA with test systems provided by Vektor-Best (Russian Federation) was used to measure the blood serum TNF- α concentrations by a microplate reader MR96 (Mindray Medical International Ltd, China) at 450 and 630 nm.

A RIBO-Prep kit provided by AmpliSens (Russian Federation) was used to isolate DNA from the venous blood. The DNA concentration and purity was measured by a spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, USA). The first-stage PCR analysis with kits provided by Litech Research and Production Company (Russian Federation) was performed on the Rotor-Gene 6000, a PCR thermocycler (Corbett Research, Australia). G308A polymorphism (rs1800629) was genotyped by allele-specific PCR with kits provided by Litech Research and Production Company (Russian Federation). Amplification products were visualized in 3% agarose gel stained with ethidium bromide under ultraviolet light.

To estimate associations of the TNF- α gene polymorphism with type 2 DM risk, the odds ratios (OR) were used for calculation as:

$$OR = (axd)/(bxc)$$

where **a** is an allele (genotype) frequency in the sample of patients, **b** is an allele (genotype) frequency in the control sample, **c** is a sum of frequencies of other alleles (genotypes) in the sample of patients and **d** is a sum of frequencies of other alleles (genotypes) in the control sample. ORs were calculated in a 95% confidence interval (CI) and were compared by Hardy-Weinberg equilibrium. OR=1 was considered as the absence of any association, the OR>1 quantifies positive association, that is, higher risk of the pathology, the OR<1 quantifies negative association of an allele or a genotype, that is, lower risk of the pathology. "DoctorStat 2013, version 1.9" software package used for statistics. Data were accepted as significant at $p < 0.05$ and smaller according to the Student's distribution.

RESULTS

Biochemical parameters of peripheral blood from patients with type 2 diabetes mellitus and persons without diabetes shown in Table 1.

As it can be seen, in the group with diabetes fasting glucose and the glycated haemoglobin (HbA1c) were respectively 2.7 and 1.6 times higher (both $p < 0.01$) than the parameters in persons without diabetes. In the diabetics, HDL cholesterol was found reduced by 22% ($P < 0.05$), concentrations of LDL cholesterol and total cholesterol were higher by 26% ($p < 0.01$) and 32% ($p < 0.05$), respectively; triglycerides were 2.76 times higher ($P < 0.01$) than in people without diabetes.

According to modern views, TNF- α inhibits the insulin-stimulated autophosphorylation of insulin receptors by tyrosine kinase in which it affects the insulin sensitivity, causes insulin resistance and abnormal glucose transport (Table 1).^{1,12}

Table 1: Biochemical parameters of peripheral blood and serum TNF- α concentration in patients with type 2 diabetes mellitus

Parameter	Non-diabetic persons	Patients with type 2 DM	P
Fasting glucose, mmol/l	3.28 ± 0.09	8.9 ± 0.4	< 0.01
HbA1c, %	5.34 ± 0.22	8.56 ± 0.39	< 0.01
Total cholesterol, mmol/l	4.25 ± 0.13	5.61 ± 0.42	< 0.02
Triglycerides, mmol/l	1.12 ± 0.24	3.10 ± 0.28	< 0.01
HDL cholesterol, mmol/l	1.19 ± 0.10	0.93 ± 0.05	< 0.02
LDL cholesterol, mmol/l	2.60 ± 0.14	3.28 ± 0.18	< 0.01
TNF- α , pg/ml	0.79 ± 0.30	5.81 ± 0.43	< 0.01

In addition, TNF- α is known to reduce activity of lipoprotein lipase and its gene expression resulting in re-store of free fatty acids in the adipose tissue, thus preventing synthesis and uptake of triglycerides by adipocytes¹¹. TNF- α has an impact on total cholesterol metabolism-enhancing its synthesis in the liver¹². The changes in the concentrations of carbohydrates and lipids in the blood of patients with type 2 DM might cause the increase in the concentrations of TNF- α ; this prompted us to determine TNF- α in peripheral blood of patients with type 2 DM.

There was a significant difference between the serum TNF- α concentrations in people with and without DM (5.81 ± 0.43 versus 0.79 ± 0.30 pg/ml) (Table 1). It is consistent with the findings of others, demonstrated the elevated TNF- α concentrations in blood serum of patients with type 2 DM^{13,14}. In our study elevation of serum TNF- α concentrations in patients with type 2 DM was shown related to abnormal carbohydrate and lipid metabolism (Figure 1).

Next, we studied frequencies of TNF- α gene G308A polymorphism in patients with type 2 DM. The genotype frequencies for the polymorphism in patients with and without type 2 DM presented in Figure 1.

Figure 1: Frequencies of gene TNF- α G308A polymorphism genotypes in patients with type 2 DM (a) and non-diabetics (b)

Our findings demonstrated that the genotype distribution for G308A polymorphic locus of the TNF- α gene in the diabetics and controls complies with Hardy-Weinberg equilibrium.

As to TNF- α gene G308A polymorphism in the sample under study, it was represented mostly by GG homozygous genotype to be registered among persons without diabetes (90.2%) and patients with type 2 DM (83.3%). GA heterozygous genotype occurred in 9.8% and 16.7% of non-diabetics and diabetics, respectively. In our study, pathological AA homozygous genotype was found neither among the diabetics nor in the controls.

As to allele frequencies, G and A alleles were found in 95.1% and 4.9%, respectively, in persons without diabetes (Figure 2). In patients with type 2 diabetes mellitus, the values were 91.7% and 8.3%, respectively; A allele occurred in the GA heterozygous variant. Thus, our findings demonstrate no significant differences ($p > 0.05$) in frequencies of alleles and genotypes of TNF α gene G308A polymorphism among non-

diabetic controls and patients with type 2 DM. In the diabetics, frequencies of A allele and GA genotype (8.3% and 16.7%, respectively) is insignificantly higher than those in the controls (4.9% and 9.8%, respectively).

Figure 2: Frequencies of gene TNF- α G308A polymorphism alleles in patients with type 2 DM (a) and the non-diabetics (b)

Our findings as per the distribution of genotypes and alleles for TNF- α gene G308A polymorphism are consistent with those from other studies in other ethnic groups^{15,16,17}.

There was no statistically significant association of carrier-ship of A allele ($\chi^2=0.62$; OR=1.76; 95% CI: 0.38-8.24) and GA heterozygous genotype ($\chi^2=0.67$; OR=1.85; 95% CI: 0.42-8.2) of TNF α gene G308A polymorphism with higher risk of type 2 DM.

In the controls, the data on G allele ($\chi^2=0.62$; OR=0.56; 95% CI: 0.13-2.63) and GG genotype ($\chi^2=0.67$; OR=0.54; 95% CI: 0.12-2.4) demonstrated their negative association with the disease, that is, G allele and GG genotype carriership in the control group was found protective and capable of reducing the risk of type 2 DM.

DISCUSSION

Type 2 diabetes mellitus is a multifactor disease resulting from the interaction between genetic and environmental factors. The genes determining the type 2 DM susceptibility exercise this interaction in cooperation with the genes underlying pathogenesis of obesity. Identification of polymorphic markers in the candidate genes products of which are involved in the pathogenesis of both obesity and type 2 DM may be of predictive value in the estimation of the disease risk. TNF- α gene with a pro-inflammatory cytokine TNF- α as its product is considered as a candidate gene.¹¹

TNF- α gene is located in the HLA III region on 6p21 chromosome.¹³ G308A polymorphism is attributable to a mutation with the single nucleotide substitution of G with A at the position 308 in the TNF- α gene promoter region. Results from many studies demonstrated that the protein synthesis is accelerated in the carriers of the polymorphism resulting in the increased production of TNF- α .^{11,14} Human TNF- α is a protein with the molecular mass of 17 kDa produced by lymphocytes, monocytes and tissue macrophages in response

to external extracellular stimulus.^{2,15} High concentrations of TNF- α blocks insulin signalling by phosphorylation of serine in the insulin receptor substrate 1 (IRS1) and induce insulin resistance in adipocytes and peripheral tissues resulting in abnormal biological effect of insulin and type 2 DM onset.^{1,15}

There are literature data on the enhanced secretion of TNF- α by monocytes-macrophages in patients with type 2 DM explaining high concentrations of TNF- α in the blood serum of patients. This may result in systemic inflammation, onset and progression of type 2 DM complications.¹⁶ The higher level of TNF- α in the blood is identified at all stages of the inflammatory process. Its biological effects depend on its concentrations; elevation plasma of the cytokine in the blood is a specific marker for abnormal carbohydrate metabolism. High concentrations of TNF- α in blood plasma were established to be associated not only with insulin resistance but also with glucose intolerance⁸; a direct correlation between TNF- α concentration and both HbA1c and glucose was found²². There is information about pro-atherogenic effects of TNF- α , as well as about its involvement in lipid metabolism and atherogenic dyslipidemia¹². TNF- α was shown to determine concentrations of triglycerides in blood plasma promoting a reduction in HDL cholesterol and an elevation in LDL cholesterol.^{17,18}

Several studies found increased concentrations of TNF- α in the blood of patients with type 2 DM.¹⁹⁻²¹ In our study, patients with type 2 DM had significantly higher concentrations of the TNF- α than healthy controls. Our findings are consistent with other authors.^{5,10,21} The increase in concentrations of TNF- α in patients with type 2 DM may be associated with the onset and progression of disease complications. Thus, in persons with diabetic nephropathy TNF- α concentrations were higher than in patients with type 2 DM without the complication suggesting higher inflammatory burden in those with diabetic nephropathy.¹⁹

In several works the high risk of type 2 DM, especially in obese subjects were linked with TNF- α gene G308A polymorphism determining the onset of metabolic disorders, such as hyperinsulinemia, dyslipidemia, arterial hypertension, Polycystic ovarian syndrome.²² Carriership of mutant 308A allele of TNF- α is considered as a predictively unfavourable factor for impaired glucose tolerance, type 2 DM and other clinical signs of insulin resistance syndrome.^{23,24}

Several meta-analyses reflect findings from the studies on the association between TNF- α gene G308A polymorphism and type 2DM risk,²⁵⁻²⁷ though the conclusions are discrepant. The discrepancy can be attributed to ethnic peculiarities, sample sizes, variety of methods used for determination of the polymorphism, as well as to the non-uniformity of groups under study by age, sex and presence or absence of the disease risk factors.

CONCLUSIONS

Analysis of allelic and genotypic frequencies for G308A polymorphic locus of TNF- α gene in our sample of patients with type 2DM and healthy controls demonstrated the statistically insignificant association between carriership of A allele and GA heterozygous genotype of TNF α gene G308A polymorphism with a higher risk of type 2 DM. G allele and GG genotype were found protective and reducing the risk of type 2 DM. Thus, it was the first study shown the nonsignificant association between the polymorphism of the TNF α promoter region G308A and type 2 DM risk among patients of Uzbek nationality. Further studies are necessary to make clear the role of TNF α gene G308A polymorphism in type 2 DM in the ethnic group.

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Conflict of Interest: Nil

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Authors' contributions

T. Saatov and Kh. Karimov provided a conception and design of the study.

S. Irgasheva, B. Zainutdinov and M. Mustafakulov dealt with the data and material collection.

S. Irgasheva, E. Ibragimova, Z. Ibragimov, T. Alimov, T. Ishanhodjaev and Z. Shamansurova performed material processing.

Competing interests

The authors have no competing interests to declare.

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