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The role of VDR and TNF gene polymorphism in cytokine regulation in type I diabetes mellitus of the Uzbek population, Samarkand, Uzbekistan

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Abstract. *Rayimova F, Dushanova G, Alikulov B, Kamalov Z, Ruzibakieva M, Nabiyeva F, Rajabov A. 2024. The role of VDR and TNF gene polymorphism in cytokine regulation in type I diabetes mellitus of the Uzbek population, Samarkand, Uzbekistan. Biodiversitas 25: 1329-1336.* After 2000, diabetes became one of the most common diseases in the world. Special attention is paid to the research to determine the variability of genetic processes in patients in the origin and development of this disease. The article is devoted to the study of the connection between the polymorphism of genotypes GG, GA, AA polymorphism BsmI of the VDR- (Vitamin D receptor) gene and GG, GA of the TNF-A-308G/A- (Tumor necrosis factor A) gene with the regulation of cytokines TNFα and IL6-Interleykin 6 in sick and healthy individuals of the Samarkand region of the Uzbek population. The analysis of the BsmI polymorphism of the VDR gene and TNF-A-308G/A indicates the involvement of certain genotypes in the Type 1 Diabetes Mellitus (T1DM) pathogenesis. The age of onset of the disease in the studied population is from 7 years, but the increase in the incidence of diseases begins from 10 years. Type 1 diabetes is more common in females. Studies of the relationship between the cytokines TNFα and IL6 show their high content depending on the genotypes GG, GA, and AA of the BsmIA polymorphism of the VDR gene and the genotypes GG and GA of the gene. TNF-A-308G/A. However, the highest rates were recorded in individuals with the AA genotype of the BsmI polymorphism of the VDR gene. The obtained results can serve as fundamental knowledge in revealing and explaining the mechanisms of cytokinin control in people with diabetes in different populations of the same nation.

Keywords: Autoimmunity, cytokines, genotypes, polymorphism, vitamin

INTRODUCTION

In the last 20 years, the influence of autoimmune processes on the development of type 1 diabetes through the regulation of genetic factors of the body that determine the synthesis of various cytokines and activation of immune reactions has been intensively studied (Hussein et al. 2012). It is known that Type 1 Diabetes Mellitus (T1DM) is a chronic multifactorial disease that develops through selective autoimmune destruction of pancreatic beta cells. Type 1 diabetes prevalence is increasing globally and has almost doubled over the past 40 years, with an incidence rate of 9.5 per 10,000 people worldwide (Atkinson et al. 2014; Miettinen et al. 2017).

Vitamin D is a strong factor contributing to the development of type 1 diabetes (Daskalopoulou et al. 2022). Many studies have examined VDR gene polymorphisms with risk of type 1 diabetes and vitamin D levels in different populations. Habibian et al. (2019) (demonstrated an association between the increasing type 1 diabetes risk and certain polymorphic variants in the VDR gene (especially BsmI and FokI) (Habibian et al. 2019). It was shown that a sufficient level of 25(OH)D in the blood serum (≥30 ng/mL) in combination with certain SNP genotypes (BsmI and TaqI) in the VDR gene in newly diagnosed type 1 diabetes patients contributed to the preservation of the function of residual β- pancreatic cells (Tapia et al. 2019). Other research revealed that SNPs in genes important for the synthesis, action, and transport of vitamin D may influence the type 1 diabetes risk of development (Norris et al. 2018; Ma et al. 2020).

Considering that today, there is a significant increase in the incidence of diabetes mellitus all over the world and several aspects of autoimmune diseases remain unsolved, the study of the immunoregulatory mechanisms of diabetes remains relevant. According to several scientific publications, in the pathogenesis of type 1 diabetes, the leading role belongs to immune-mediated mechanisms, which in turn are initiated by various trigger factors, which ultimately lead to immune dysregulation both at the local and systemic levels with the launch of the body's autoimmune response mechanism (Li et al. 2014).

Cytokines are crucial in the immunopathogenesis of autoimmune diabetes (Isailovic et al. 2015). These include tumor necrosis factor α (Tumor Necrosis Factor α (TNFα)) (Pan et al. 2022). Under physiological conditions, TNFα manifests itself as an immunoregulator, is involved in the proliferation and differentiation of various cell types, influences cell apoptosis, and stimulates the production of various cytokines (van Loo et al. 2023). Experimental data have shown that the inflammatory autoimmune process in the pancreas at the onset of the disease is associated with increased production of proinflammatory cytokines IL3, TNFα, and INFγ.

TNF-α is key in inflammatory cascades at both systemic and local levels (Chen et al. 2017; Megha et al. 2021). It is directly involved in the pathogenesis of many systemic diseases. More importantly, adequate levels of TNF-α are beneficial for maintaining key homeostatic functions of normal cells, such as cell proliferation, necrosis, and apoptosis (Liu et al. 2023).

Interleukin 6 (IL6 gene 7p15-21-q21) is a cytokine family member with proinflammatory properties. The cytokine is produced primarily by cells of the immune system: monocytes, lymphocytes, macrophages, endothelial cells, and microglia, and is also produced by several non-immune cells (Wassmann et al. 2004). IL6 activates endothelial cells and ensures the collection of leukocytes near the vessel walls (Villar-Fincheira et al. 2021). It is assumed that such IL6 activity may gradually destroy pancreatic β-cells (Szableweski 2014). IL-6 signals through a type 1 cytokine receptor complex on the cell membrane consisting of the IL-6Rα ligand-binding chain (CD126) and the signaling component gp130 (also called CD130) (Hussein et al. 2012; Liu et al. 2023). It is possible that IL-6 also has anti-inflammatory effects, which are most likely due to its classical signaling (Schaible et al. 2010; Gupta et al. 2018).

Based on the presented data, it was necessary to study the relationship between gene polymorphism and the regulation of cytokines in patients with type 1 diabetes of Uzbek population in Samarkand region.

MATERIALS AND METHODS

Sample collection

64 patients (29 men and 35 women) and 138 healthy (76 men and 62 women) individuals in the Samarkand region, aged 10 to 40 years, were studied. The study group of patients with type 1 diabetes included patients registered at the Samarkand region's endocrinology center. An analysis of the polymorphism of the VDR and TNF genes was carried out in sick and healthy individuals; the studies were conducted in laboratory of molecular genetics of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan. Patients with newly diagnosed type 1 diabetes mellitus included in the study did not have significant gender differences compared to the control group. All enrolled patients participated in the study with their consent.

DNA extracti200on

The material for DNA extraction was venous blood from the cubital vein with a volume of 3-5 mL (Beckton-Dickinson vacutainers were used for blood collection) with an anticoagulant/preservative 15% tripotassium EDTA (Ethylenediaminetetraacetic Acid). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding +4°C. Therefore, to obtain genomic DNA, a two-step method of blood cell lysis was used by double centrifuging the entire volume of whole blood in RCLB buffer (Red-Cells Lysis Buffer) at a speed of 1500 rpm for 15-20 minutes, and erythrocyte lysis was carried out. Using RCLB causes osmotic shock to red blood cells, leading to their swelling and further destruction.

Genotyping by RFLP-PCR

Genotyping of polymorphic gene regions was carried out using the Polymerase Chain Reaction (PCR) method with allele-specific primers (NPF "Litekh," Moscow). This polymorphism was determined according to the PCR-RFLP method. PCR amplification was performed using primers for VDR BsmI rs1544410: forward: 5'-AACCAGCGGGA AGAGGTCAAGGG-3' and reverse: 5'-CAACCAAGACT ACAAGTACCGCGTCAGTGA-3' and for the TNF-A gene -308G/A: forward 5'-GACAAGCCTGTAGCCCATGT-3' and the reverse 5'- GGAGGTTGACCTTGGTCTGG -3' (Darawi et al. 2013).

The PCR mixture contained 100 ng of DNA template, 20 nmol of each primer, 1.5 mM MgCl2, 0.2 mM dNTP, and 1.0 units. AmpliTaq DNA polymerase, as well as amplification conditions and electrophoretic detection of reaction products in an agarose gel, these SNPs are previously confirmed and have a minor allele frequency of 1% or more (NCBI dbSNPdatabase, http://www.ncbi.nlm.nih.gov/projects/SNP/index.html).

Statistical processing of genotyping data

The collected data were processed using statistical software packages Arlequin 2006 (version 3.5.2.2.), Excel 2003, and SISA. The distribution of genotypes at the studied polymorphic loci was studied using logistic regression analysis and testing for compliance with Hardy-Weinberg equilibrium using Fisher's exact test. The correspondence of patients and controls by gender and age was considered.

Moreover, statistically significant at $p<0.05$ was considered a difference. Identification of amplification products and their distribution concerning the length marker was carried out in ultraviolet light (310 nm) after electrophoresis for 15 minutes or in 10% PAAG 29:1 at a voltage of 300 V (in both cases, the range was 3-4 cm) and staining ethidium bromide. Digestion of plasmid pUC19 with restriction enzyme Msp1 was used as a length marker (Li et al. 2017).

Determination of cytokine concentration by ELISA (Enzyme-Linked Immunosorbent Assay)

The cytokines concentration was determined by ELISA using commercial kits from JSC Vector Best in the concentration range for TNF-α pg/mL, IL-6 5.6-300 pg/mL (Li et al. 2022).

Statistical data processing

The Microsoft Office Excel-2003 software package, including built-in statistical processing functions, was used to process the obtained data statistically. With the help of this program, operations were performed to determine the arithmetic mean value and deviations of the indicators using the appropriate formulas, and transfer digital data to the form of graphs.

RESULTS AND DISCUSSION

When analyzing the results obtained as part of genotyping, it was revealed that allele A was found 1.64 times more often in the group of patients compared to the control group, with indicators OR = 1.978, 95%CI = 1.091 >1.978>3.585, $\chi 2$ = 5.13 (p = 0.02) (Table 1). Allele G was more common in the group of healthy individuals, with OR = 0.506, 95% CI = 0.279>0.506>0.917, χ^2 = 5.13 (p = 0.02). When analyzing genotypes, the homozygous genotype GG of the vitamin D receptor gene BsmI rs1544410 showed significant differences in the frequency of occurrence since this genotype was 1.5 times more common in the group of practically healthy individuals with indicators OR = 0.429, 95%CI = 0.204>0.429>0.902, χ^2 = 5.062 (p = 0.024451). No significant differences were found in this sample for the heterozygous genotype GA and homozygous genotype AA of the vitamin D receptor gene BsmI rs1544410. Thus, in this sample, when studying the nature of the distribution of alleles and genotypes of the vitamin D receptor gene BsmI rs1544410, the protective allele G and the protective genotype GGa were significant, as well as the allele A, which is a significant predisposing marker, however, genotypes with the presence of this allele did not reach their true significance.

A comparative analysis of the genotypes of the BsmI polymorphism of the VDR gene by different age groups of patients with T1DM is presented in Table 2. In Samarkand patients with T1DM of the GG genotype of the BsmI polymorphism, a statistically significant difference was shown in people over 10 years old; in people with the GG genotype 7-10 years old, the indicators do not have a statistically significant difference (P≤0.0001). In persons with the GA genotype, a statistically significant difference is also observed in persons over 10 years of age. A statistically significant difference is not observed in persons 7-10 and over 15 years. The statistical analysis shows that in individuals with the AA BsmI genotype of the VDR gene polymorphism, there is no significant difference between the onset of the disease at the age of 7-10 years, 10-15, and over 15 years. The group older than 10 years had statistically significant differences (P≤0.0001). Thus, the studies show the involvement of adolescent children over 10 years of age in the disease.

In a comparative study of the distribution of allele frequencies and genotypes of polymorphic markers of the TNFα -308G/A gene in groups of patients with type 1 diabetes, in individuals of the Uzbek population living in the Samarkand region and controls (Table 3), a statistically significant increase in the frequency of the A allele in patients with compared with the control group (12.5% and 6.57%, respectively; OR = 2.033; 95% CI: 1.053>2.033 >3.924; χ^2 = 4.62 (p = 0.03)). The G allele of the studied polymorphism was found significantly less frequently compared to the control group (87.5% and 93.43%, respectively; OR = 0.492; 95% CI: 0.255>0.492>0.949; χ^2 = 4.62 (p = 0.031593)).

A comparative analysis of TNFα -308G/A genotypes in the distribution pattern of GG genotypes revealed significant differences between patients with type 1 diabetes in individuals of the Uzbek population living in the Samarkand region and the control group (75.0% and 86.87%, respectively; OR = 0.453; 95% CI: 0.225 >0.453> 0.913; χ^2=5.062). When analyzing the heterozygous GA genotype, differences were identified between the frequency of occurrence in patients and the control group (25.0% and 13.13%, respectively; OR = 2.205; 95% CI: 1.095>2.205> 4.441; χ^2 = 5.062). As already described above, a significant difference was found in the frequency of occurrence of allele A, the studied polymorphism TNFα -308G/A. However, the genotypic analysis of the homozygous AA genotype was not registered.

The data obtained indicate that the TNF-308(G/A) polymorphism significantly contributes to susceptibility to the disease and is a significant prognosis factor in patients with type 1 diabetes in the Uzbek population living in the Samarkand region.

When comparing the analysis of the genotypes of the TNFα -308G/A polymorphism with different age groups of T1DM patients (Table 4), in children with T1DM with the GG genotype, there were statistically significant differences in those over 10 years of age (P≤0.0001). There were no statistically significant differences in the age subgroups in persons with the GG genotype 7-10 and over 15. Also, in individuals with the GA genotype, adolescents in the age group over 10 years old had statistically significant differences (P≤0.0001) compared with other age subgroups.

Table 1. Distribution of genotypes of the vitamin D receptor gene BsmI rs1544410 in patients with type 1 diabetes

Genotype	Patients, n = 64	Patients, %	Genotype	Control, n = 54	Control, %	χ^2*	RR (95% CI)**
G	85	66.41	G	86	79.63	5.132 (p = 0.023483)	0.279>0.506>0.917
A	43	33.59	A	22	20.37		1.091>1.978>3.585
GG	27	42.19	GG	34	62.96	5.062 (p = 0.024451)	0.204>0.429>0.902
GA	31	48.44	GA	18	33.33	2.752 (p = 0.09715)	0.889>1.879>3.972
AA	6	9.38	AA	2	3.70	1.491 (p = 0.222136)	0.52>2.69>13.914

Note: *χ^2: Pearson reliability indicator, **RR: Relative Risk

Table 3. Distribution of frequencies of alleles and genotypes of the TNF-A -308G/A gene in patients with type 1 diabetes, in individuals of the Uzbek population living in the Samarkand region, Uzbekistan

Genotype	Patients, n = 64	Patients, %	Genotype	Control, n = 168	Control, %	χ^2*	RR (95% CI)**
G	112	87.50	G	370	93.43	4.62 (p = 0.031593)	0.255>0.492>0.949
A	16	12.50	A	26	6.57		1.053>2.033>3.924
GG	48	75.00	GG	172	86.87	5.062 (p = 0.024463)	0.225>0.453>0.913
GA	16	25.00	GA	26	13.13	5.062 (p = 0.024463)	1.095>2.205>4.441
AA	0	0.00	AA	0	0.00		

Note: * χ^2: Pearson reliability indicator, **RR: relative risk

Table 2. BsmI genotypes in children of age groups

Genotypes	Frequencies (%)	RR (95%CI)*	P-value
GG			
7-10 years	3/27 (11.11%)	0.87 (0.41-1.82)	0.85
10-15 years	4/27 (14.81%)		
≥10 years	1/27 (3.7%)	0.20 (0.10-0.39)	≤0.0001
≥15 years	15/27 (55.55%)		
GA			
7-10 years	6/31 (19.35%)	0.85 (0.26-2.71)	1.0
10-15 years	6/31 (19.35%)		
≥10 years	1/31 (3.22%)	0.17 (0.20-0.30)	≤0.0001
≥15 years	18/31 (58.06%)		
AA			
7-10 years	3/6 (50%)	0.60 (0.33-1.07)	0.11
10-15 years	2/6 (33.33%)		
≥10 years	2/6 (33.33%)	0,28 (0,17-0,47)	≤0.0001
≥15 years	4/6 (66.66%)		

Note: *RR: relative risk

Table 4. TNFα -308G/A genotypes in children of age groups

Genotypes	Frequencies (%)	RR (95%CI)*	P-value
GG			
7-10 years	6/48 (12.5%)	0.81 (0.21-2.54)	0.91
10-15 years	8/48 (16.6%)		
≥10 years	4/48 (8.33%)	0.15 (0.19-0.28)	≤0.0001
≥15 years	28/48 (58.33%)		
GA			
7-10 years	3/16 (18.75%)	0.81 (0.38-1.74)	0.81
10-15 years	4/16 (25%)		
≥10 years	2/16 (12.5%)	0.21 (0.15-0.38)	≤0.0001
≥15 years	9/16 (56.25%)		

Note: *RR: relative risk

To clarify the nature of the development of type 1 diabetes, we analyzed the levels of cytokines TNF-α and IL-6 in individuals with type 1 diabetes. The conducted studies show that analysis of the level of proinflammatory cytokines (TNF-α and IL-6) in type 1 diabetes revealed a significant difference (P<0.05) in type 1 diabetes in the content of these cytokines in peripheral blood serum (Figure 1). Thus, the concentration of TNF-α in the peripheral blood of patients was 56.7±4.8 pg/mL, while in the control group this figure was 16.3±1.76 pg/mL. The data presented show a 3.5-fold increase in serum TNF-α levels in patients with type 1 diabetes compared to control values.

TNF-α is a cytokine involved in systemic inflammation. Plasma TNF-a levels are associated with various diabetes risk factors such as dyslipidemia, obesity, and inflammation. It plays a fundamental role in the destruction of beta cells mediated by immune cells; therefore its significantly (P<0.05) high content in patients with type 1 diabetes (56.7±4.8 pg/mL) compared with patients with diabetes Type 2 (35.27±3.1 pg/mL) indicates the predominance of the immune-mediated destructive process of beta cells in the pathogenesis of type 1 diabetes compared to type 2 diabetes, the pathogenetic mechanisms of which are largely metabolic disorders.

However, TNF-α is inhibited by IL-6, which to some extent plays a protective role (Shcherbak 2011). Therefore, the comparative content of IL-6 was studied among the examined patients with type 1 and type 2 diabetes and the control group. IL-6, in terms of the direction of action of its properties, can be considered both a pro- and anti-inflammatory cytokine (Wassmann et al. 2004). On the one hand, IL-6, to some extent, reduces the production of proinflammatory cytokines by macrophages. On the other hand, it induces the production of acute phase proteins that activate the production of corticosteroids, thereby leading to the activation of T lymphocytes by antigen-presenting cells, enhancing B cell proliferation and enhancing the formation of immunoglobulins (Szablewski 2014). In patients with type 1 diabetes, the level of IL-6 was significantly higher than the control values of this cytokine. Thus, the level of IL-6 in the group of patients with type 1 diabetes was 34.1±1.3 pg/mL, while in the control group, this figure was 5.2±0.8 pg/mL (P<0.05). From the presented data, it is clear that the level of IL-6 in the group of patients with type 1 diabetes was 6.5 times higher than in the control group. Therefore, considering that the level of increase in TNF-a compared to the control was 3.5 times, we can assume that in this cohort of patients, IL-6 apparently showed its proinflammatory properties to a greater extent.

To better consider the relationship between the cytokines studied, the ratio between TNF-α and IL-6 (TNF-α/IL-6) was examined (Figure 2). Thus, in the control group, this ratio was 3.1, in type 1 diabetes -1.6, in type 2 diabetes -1.5. From the presented data, it is clear that the initiation of the inflammatory process in type 1 and type 2 diabetes occurs more from the side of adaptive immunity, that is, due to a greater increase in the level of IL-6 compared to the level of increase in TNF-a, that is, due to increased T-cell activation and chronicity of the immune-mediated inflammatory process. Thus, the changes that develop against the background of diabetes mellitus with an increase in the level of proinflammatory cytokines include pronounced immune-mediated inflammation of pancreatic β-cells due

to the proliferation of endothelial cells and the attraction of a large number of different lymphoid cells to these cells, with the formation of monocyte-macrophage accumulations. It is believed that the most potent proinflammatory cytokine is IL-6, which can induce the production of other cytokines and interact synergistically with them, affecting endothelium, chondrocytes, osteoclasts, and phagocytic cells (Burrack et al. 2017).

Table 5. The level of activity of cytokines TNFα, IL6 in patients with type 1 diabetes in the Samarkand region, Uzbekistan

Genotype	TNFα, pg/mL, n = 64	Control	IL6, pg/mL, n = 64	Control
BsmI				
GG n = 27	52.8±3.8*	16.3±1.76	29.3±2.4	5.2±0.8
GA n = 31	54.3±4.2		33.4±2.8	
AA n = 6	62.3±1.8		39.7±1.3	
TNFα-308G/A				
GG n = 48	55.3±5.6	16.3±1.76	32.2±2.7	5.2±0.8
GA n = 16	58.1±4.4		37.1±1.1	

Note: *Significance of the difference between the studied group and the control (p≤0.001)

Table 6. The level of activity of cytokines TNFα, IL6 in women with type 1 diabetes in the Samarkand region, Uzbekistan

Genotype	TNFα, pg/mL, n = 64	Control	IL6, pg/mL, n = 64	Control
BsmI				
GG n = 20	54.6±3.8*	16.3±1.76	29.3±2.4	5.2±0.8
GA n = 17	55.1±4.2		33.4±2.8	
AA n = 4	63.3±1.8		39.7±1.3	
TNFα -308G/A				
GG n = 23	55.1±5.6		32.2±2.7	
GA n = 14	57.1±4.4		37.1±1.1	

Note: *Significance of the difference between the studied group and the control (p≤0.001)

Table 7. The level of activity of cytokines TNFα, IL6 in men with type 1 diabetes in the Samarkand region, Uzbekistan

Genotype	TNFα, pg/mL, n = 64	Control	IL6, pg/mL, n = 64	Control
BsmI				
GG n = 7	51.2±3.8*	16.3±1.76	29.3±2.4	5.2±0.8
GA n = 14	54.3±4.2		33.4±2.8	
AA n = 2	61.2±1.8		39.7±1.3	
TNFα -308G/A				
GG n = 11	56.3±5.6		32.2±2.7	
GA n = 10	59.1±4.4		37.1±1.1	

Note: *Significance of the difference between the studied group and the control (p≤0.001)

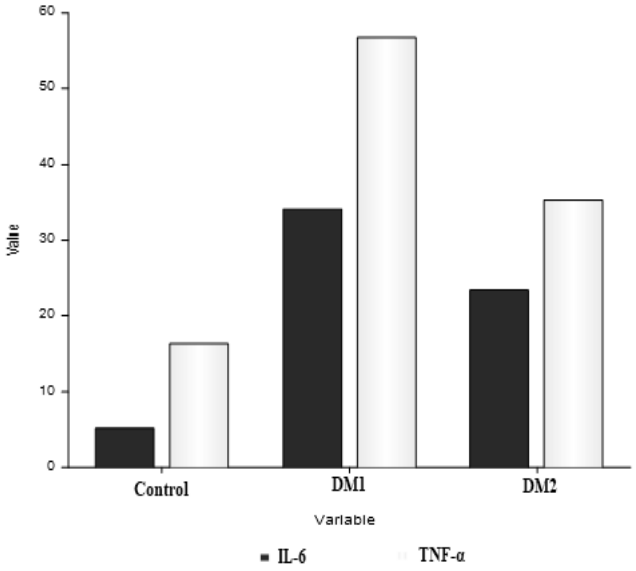

Figure 1. Level of main proinflammatory cytokines in type 1 and type 2 diabetes

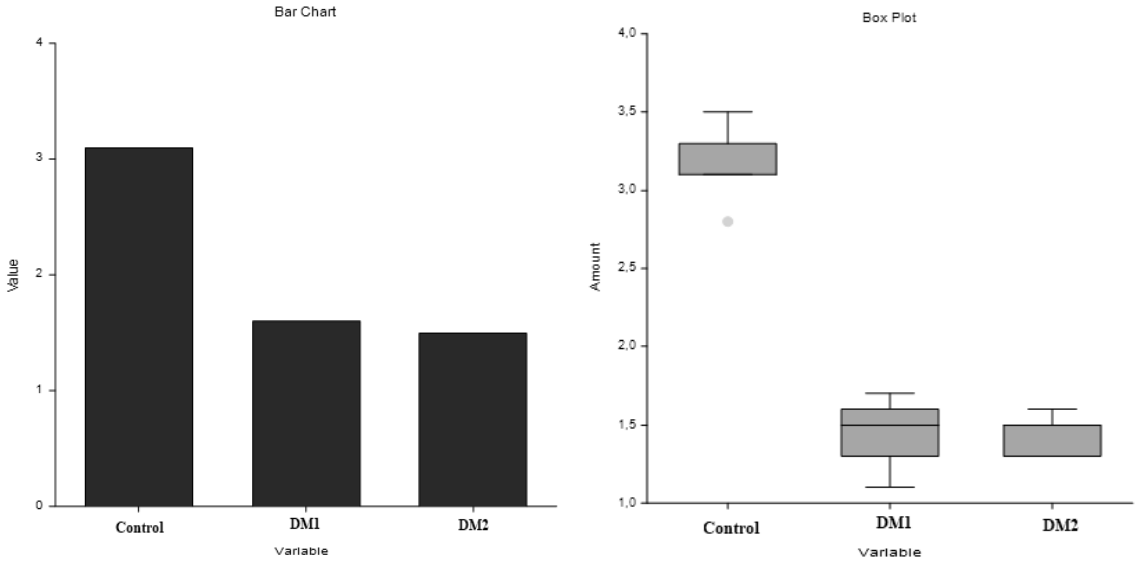

Figure 2. The ratio of the main proinflammatory cytokines in type 1 and type 2 diabetes

We carried out a comparative characterization of the level of activity indicators of the cytokines TNFα, IL6 in patients with type 1 diabetes in the Samarkand region with genotypes GG, GA, AA BsmI of the VDR gene and GG, GA genotypes of the TNFα -308G/A gene in the general population, the data are presented in Table 5 According to Table 5, it can be seen that the level of activity indicators of cytokines TNFα, IL6 in patients with type 1 diabetes in the Samarkand region, depending on the polymorphism of the BsmI gene VDR polymorphism TNFα -308G/A, was shown in individuals with genotype GG, GA and AA polymorphism of the BsmI gene VDR, high concentrations of TNFα pg/mL are observed, but the highest concentrations are observed in individuals with the AA genotype (52.8±3.8, 54.3±4.2, 62.3±1.8, 16.3±1, 76, p≤0.001) compared to the control group. In individuals with genotypes GG and GA of the TNFα -308G/A polymorphism, an increase in the amount of TNFα pg/mL is also observed (55.3±5.6, 58.1±4.4, 16.3±1.76, p≤0.001) high concentrations of which were observed in individuals with the GA genotype. Analysis of cytokine levels in individuals with the GG, GA, and AA genotypes of the BsmI polymorphism of the VDR gene showed high levels of cytokines in individuals with type 1 diabetes, but the highest concentrations were observed in individuals with the AA genotype compared to controls (29.3±2.4, 33.4±2.8, 39.7±1.3, 5.2±0.8, p≤0.001).

According to the data presented in Table 5 and Figure 3, in the general population with type 1 diabetes, the gene with GG genotypes n=27 had high concentrations of TNFα pg/mL compared to the control group, but the highest concentrations were in individuals with the AA genotype of the VDR gene BsmI polymorphism (Figure 3).

Analysis of gender differences in the level of cytokines TNFα and IL6 depending on the genotypes GG, GA, and AA of the BsmI polymorphism of the VDR gene and TNFα -308G/A in women is presented in Table 6 and Figure 4. According to Table 6, it can be seen that in individuals with genotypes GG, GA and AA of the BsmI polymorphism of the VDR gene, an increase in the amount of TNFα cytokines was observed compared to the control group (54.6±3.8, 55.1±4.2, 63.3±1.8, 16.3±1.76, p≤0.001). In individuals with the TNFα -308G/A gene polymorphism with the GG and GA genotypes, an increase in the number of cytokines was observed (55.1±5.6, 57.1±4.4, 16.3±1.76, p≤0.001) compared with the control group. In women with the GG, GA, and AA genotypes of the BsmI polymorphism of the VDR gene, an increase in IL6 cytokines was observed (29.3±2.4, 33.4±2.8, 39.7±1.3, 5.2±0.8, p≤0.001) compared with the control group. With the GG and GA genotypes, an increase in the amount of IL6 cytokines was observed (32.2±2.7, 37.1±1.1, 5.2±0.8, p≤0.001) compared to the control.

Analysis of the content of cytokines TNFα and IL6 in men with type 1 diabetes in the Samarkand region of the BsmI polymorphism of the VDR gene and TNFα -308G/A in males is presented in Table 7. According to the data in Table 7, it can be seen that in individuals with genotypes GG, GA, and AA, there is an increase in the number of TNFα cytokines compared to the control (51.2±3.8, 54.3±4.2, 61.2±1.8, 16.3±1.76, p≤0.001) but the highest rates are observed in individuals with the AA genotype. Individuals with genotypes GG, GA, and AA also have high levels of IL6 compared to the control group (29.3±2.4, 33.4±2.8, 39.7±1.3, 5.2±0, 8, p≤0.001), individuals with the AA genotype have the highest levels of IL6 compared to individuals with the GG and GA genotypes (Figures 5A-5C).

Individuals with genotypes GG and GA of the TNFα -308G/A gene polymorphism have high TNFα levels compared to controls (56.3±5.6, 59.1±4.4, 16.3±1.76, p≤0.001). Analysis of IL6 content with these genotypes of the TNFα -308G/A gene polymorphism showed high levels of this cytokine compared to the control group (32.2±2.7, 37.1±1.1, 5.2±0.8, p≤ 0.001).

Considering the diverging data in different populations/ ethnic groups, we investigated the prevalence of type 1 diabetes in the age group from 4 to 40 years. The relationship of the age factor with the onset of the disease and the connection between the VDR and TNFα -308G/A gene polymorphism with the regulation of TNFα and IL6 cytokines in the pathogenesis of the disease, study the nature of the pathogenesis of an autoimmune nature. The conducted studies prove that in the studied sample when studying the nature of the distribution of alleles and genotypes of the vitamin D receptor gene BsmI rs1544410, the protective allele G and the protective genotype GG, as well as the allele A, which is a significant predisposing marker, were significant, but genotypes with the presence of this allele did not reach its true significance in a given sample. The study of polymorphism -308(G/A) TNFα makes a significant contribution to susceptibility to the disease and is a significant prognosis factor in patients with type 1 diabetes in the Uzbek population living in the Samarkand region.

Studies of the factors of onset of the disease depending on the genotype GG, GA, and AA of the BsmI polymorphism of the VDR gene and GG, GA of the TNFα -308G/A gene show the age of onset of the disease from 7 years, but the number of patients with type 1 diabetes increases significantly in the population from 10 years, and more the number of cases of illness is among girls. An analysis of gender differences in the incidence of type 1 diabetes showed that in the population of those examined, type 1 diabetes is more common often in females, that is, in young girls.

Analysis of the relationship between the studied cytokines showed the relationship between TNFα and IL-6 (TNFα/IL-6). Thus, in the control group it was 3.1, in type 1 diabetes - 1.6, in type 2 diabetes -1.5. The presented data show that the initiation of the inflammatory process in type 1 and type 2 diabetes occurs more on the part of the adaptive immune system, that is, due to a greater increase in the level of IL-6 compared to the level of increase in TNFα, that is, due to increased T-cell activation and chronicity of immune-mediated inflammatory process. Studies of the relationship between the genotype GG, GA, and AA polymorphism BsmI of the VDR gene and GG, GA of the TNFα -308G/A gene with indicators of the cytokines TNFα and IL6 prove the involvement of immune reactions in the pathogenesis of type 1 diabetes. Females with the AA genotype of the BsmI polymorphism of the VDR gene have a high risk of developing the autoimmune nature of type 1 diabetes.

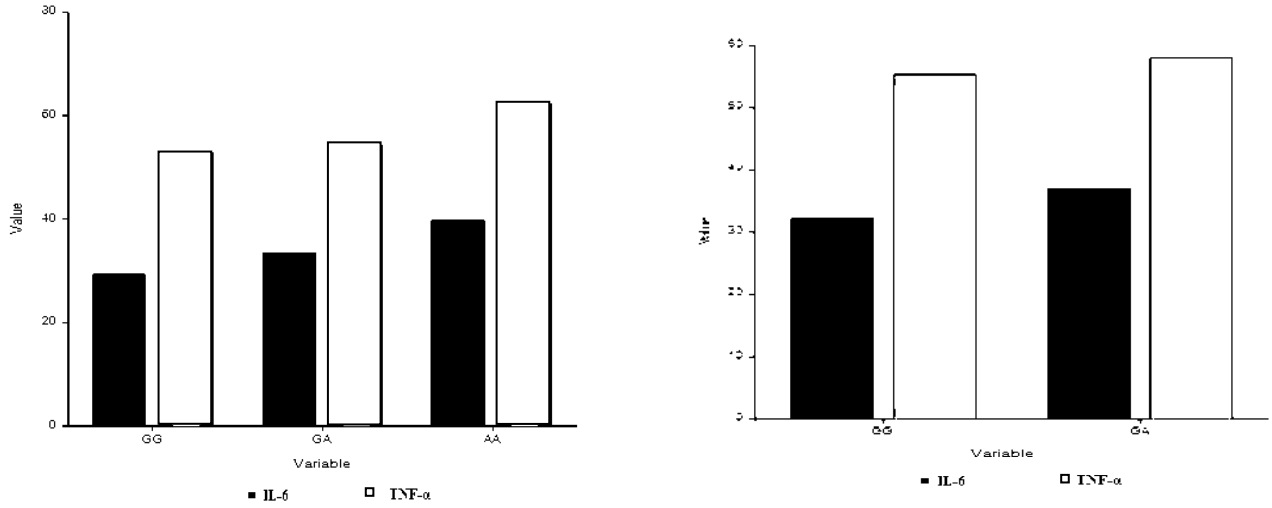

Figure 3. The content of TNFα and IL6 in individuals with genotypes GG, GA and AA of the BsmI polymorphism of the VDR gene and in individuals with genotypes GG, GA of the TNFα -308G/A gene polymorphism

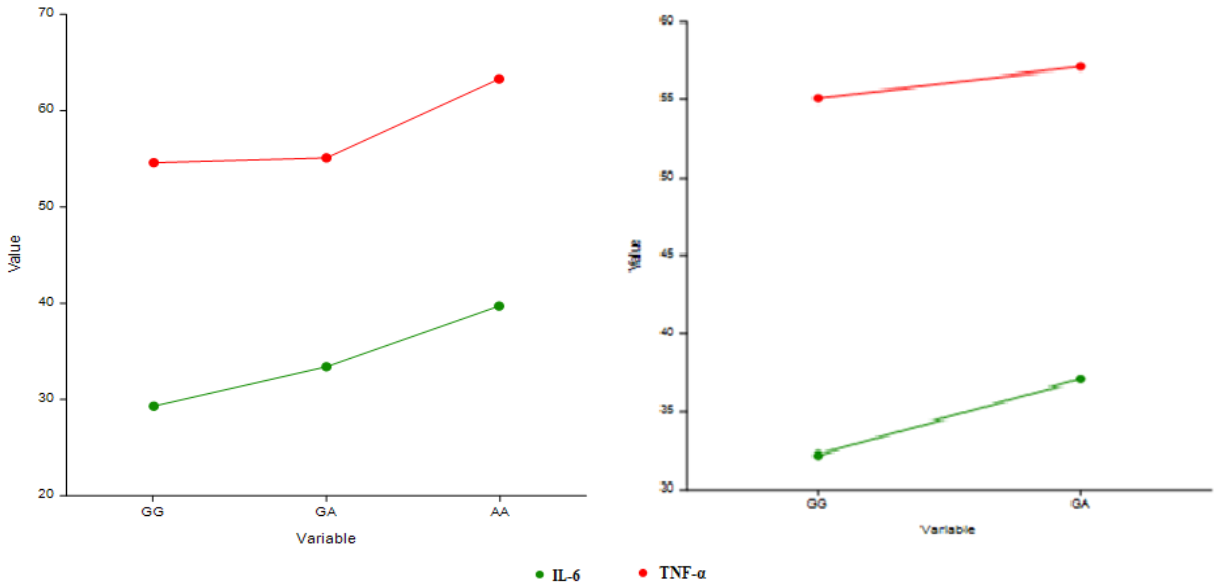

Figure 4. The content of TNFα and IL6 in individuals with genotypes GG, GA and AA of the BsmI polymorphism of the VDR gene and in individuals with genotypes GG, GA of the TNFα -308G/A gene polymorphism in females

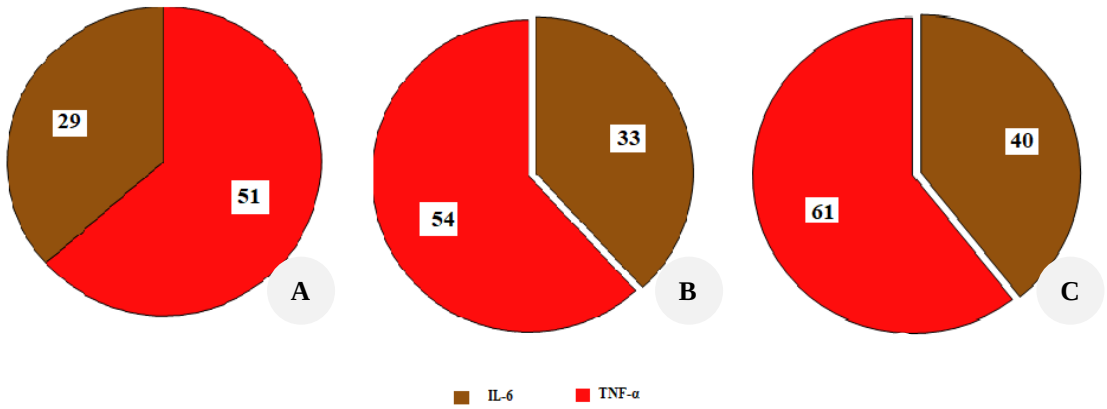

Figure 5. TNFα and IL6 content in individuals with: A. GG, B. GA, C. AA genotypes of the BsmI polymorphism of the VDR gene in males (pg/mL)

In conclusion, determined of distribution of alleles and genotypes of the vitamin D receptor gene BsmI rs1544410 were significant: the protective allele G and the protective genotype GG, as well as the allele A, which is a significant predisposing marker, but genotypes with the presence of this allele, did not reach their true significance in this sample. The study of polymorphism -308(G/A) TNFα makes a significant contribution to susceptibility to the disease and is a significant prognosis factor in patients with type 1 diabetes in the Uzbek population living in the Samarkand region. Studies of the factors of onset of the disease depending on the genotype GG, GA, and AA of the BsmI polymorphism of the VDR gene and GG, GA of the TNFα -308G/A gene show the age of onset of the disease from 7 years, but the number of patients with type 1 diabetes increases significantly in the population from 10 years. The presented data show that the initiation of the inflammatory process in type 1 and type 2 diabetes occurs more from the adaptive immune system. Studies of the relationship between the genotype GG, GA, and AA polymorphism BsmI of the VDR gene and GG, GA of the TNFα -308G/A gene with indicators of the cytokines TNFα and IL6 prove the involvement of immune reactions in the pathogenesis of type 1 diabetes. The study's results may be important in selecting aspects and processes that should be taken into account in managing cytokinin in people with diabetes in different populations.

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