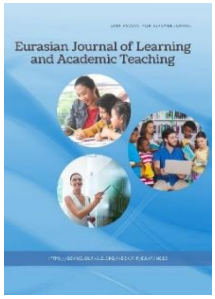

The Role Of Il6 Gene C174g Polymorphism In The Progression Of Diabetic Foot Syndrome

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ABSTRACT

The criteria for risk stratification of the formation of diabetic foot syndrome in patients with diabetes mellitus based on the carriage of the interleukin 6 gene were studied.
The clinical group included 96 patients aged 40 to 75 years with diabetes mellitus complicated by diabetic foot syndrome. The control group consisted of 83 healthy individuals. The results obtained by the authors demonstrate that the presence of the C/G genotype of the C174G polymorphism of the IL6 gene has a clear correlation with predisposition to the development of diabetic foot syndrome. In order to effectively prevent the development of diabetic foot syndrome, it is advisable to recommend genotyping of the C174G polymorphism in the IL6 gene.

Keywords: diabetic foot, gene polymorphism, diabetes mellitus

Actuality of the problem. Diabetes mellitus (DM) is a chronic metabolic disease. More than 382 million people worldwide suffer from diabetes, and the prevalence of type 2 diabetes mellitus (T2DM) in children and adolescents is increasing worldwide in parallel with rising rates of obesity [1]. By 2030, diabetes may become the seventh leading cause of human death [2,6,8,9]. DM is associated with microvascular and macrovascular complications, which are considered one of the leading causes of morbidity and mortality. Chronic complications, especially microvascular complications (diabetic retinopathy, nephropathy, foot disease, neuropathy), are the main dangerous outcome of this disease [4,11]. Chronic inflammatory processes are involved in the development of diabetic microvascular complications. Inflammatory cytokines, including interleukin-6 (IL-6), play an important role in the pathogenesis of T2DM and its complications [3,15].

Recently, single nucleotide polymorphism (SNP) of the IL-6 gene has attracted increasing attention. Significant IL-6 gene sequence variation 174G/C is widespread with varying serum IL-6 levels in genetically susceptible individuals [4]. A G/C polymorphism at position-174 in the IL-6 promoter region (rs1800795) has been found to correlate with retinopathy, nephropathy [5,12], elevated albumin-to-creatinine ratio, as well as poor glycemic control and hyperlipidemia in type 1 diabetes. diabetes mellitus (DM1) [6,14]. However, the results of current studies on the involvement of this SNP rs1800795 in diabetic complications in T2DM are conflicting. It remains unclear whether the IL-6 rs1800795

gene polymorphisms can serve as genetic predictors of the progression of complications in T2DM and help identify patients at high risk of diabetic complications, thereby helping them in individualized treatment [7].

To implement the basic principles of modern personalized medicine, it is relevant to study the genetic aspect of multifactorial diseases, which include diabetes mellitus and its complications, in order to identify numerous genetic polymorphisms that should be taken into account in combination with modifiable and non-modifiable non-genetic factors [9].

Aim of the research. Analysis of the distribution of the C174G polymorphism of the IL6 gene among patients with DFS and to identify a possible association of this polymorphism with the risk of developing DFS in patients with diabetes mellitus in the Uzbek population.

Material and methods of the research. To solve these problems, we conducted a genetic study on 96 patients who were treated in the departments of the clinic of the Andijan State Medical Institute, who made up the main group. Inclusion criteria were: presence of type 2 diabetes mellitus, patient age from 40 to 75 years. The control group consisted of 83 healthy individuals.

Determination of allelic and genotypic variants of polymorphism in the IL6 gene (C174G) was carried out in the Department of Molecular Medicine and Cellular Technology at the Republican Scientific and Practical Medical Center for Hematology of the Ministry of Health of the Republic of Uzbekistan. The main method of molecular genetic research was PCR analysis. Genomic DNA was isolated from peripheral blood lymphocytes of patients using the AmpliPrime RIBO-prep isolation kit (Interlabservice LLC, Russia). The study was carried out using quantitative real-time PCR analysis (Real-Time PCR). Amplification was carried out using a thermal cycler for real-time PCR analysis - Rotor Gene Q, (Quagen, Germany). To determine genetic markers, test systems from the Synthol company (Russia) were used according to the manufacturer's instructions. To compare the distribution of genotypes in the experimental and control groups, as well as the correspondence of this distribution to the Hardy-Weinberg equilibrium, the $\chi 2$ - Pearson test was used. To establish the risk of developing DFS, the odds ratio (OR) and 95% confidence interval (CI) were calculated. For statistical processing of the results obtained, the application package "OpenEpi, 2009", VERSION 9.2 was used. Statistical analysis followed the International Committee of Medical Journal Editors (ICMJE) guidelines and the Statistical Analysis and Methods in the Published Literature (SAMPL) guidelines. Nominal data were described indicating absolute values and percentages.

The results obtained and their discussion. During the study in the main group, the actual distribution of genotypes of the C174G polymorphism of the IL6 gene corresponded to those expected at Hardy-Weinberg equilibrium (HWE) ($p < 0.05$). And also, in the control group, the observed distribution of genotypes of the C174G polymorphism of the IL6 gene also did not deviate from the Hardy-Weinberg equilibrium (HWE) ($p < 0.05$). The frequencies of G and C alleles, respectively, were: 0.76/0.24 - in the patient group and 0.89/0.11 in control group (Tables 1, 2).

In the group of patients, the observed distribution of the homozygous genotype G / G was insignificantly increased in comparison with the theoretical one (0.59 versus 0.57, respectively; $\chi 2=0.09$; $p=0.207$). On the contrary, the observed frequency of the unfavorable heterozygous genotype G/C is statistically insignificantly lower compared to the expected one (0.32 versus 0.37, respectively; $\chi 2=0.88$; $p=0.207$). The mutant genotype C/C in the studied groups was found in insignificant quantities compared to the expected ones - 0.08 versus 0.06 with $\chi 2=1.54$; $p =0.207$.

Table 1.

Distribution of alleles and genotypes of the C174G polymorphic variant of the IL6 gene in HWE in the main group.

Alleles	Allele frequency

G	0,76				
C	0,24				
Genotypes	**Genotype frequency**		**χ2**	**p**	**df**
	observed	**expected**			
G /G	0,59	0,57	0,09		
G /C	0,32	0,37	0,57		
C /C	0,08	0,06	0,88		
Total	1	1	1,54	0,207	1

In the control group, the observed and expected genotype frequencies were almost identical. In particular, the indicators of the homozygous genotype G/G corresponded to 0.81 versus 0.79 (χ2=0.02 and p=0.239), the heterozygous genotype G/C was slightly lower - 0.17 versus 0.19 (χ2=0.26; p=0.239). The homozygous genotype C/C was also almost the same – 0.02 versus 0.01 (χ2=1.07; p=0.239) (Table 2).

Table 2.
Distribution of alleles and genotypes of the C174G polymorphic variant of the IL6 gene in HWE in the control group.

Control group					
Alleles	**Allele frequency**				
G	0,89				
C	0,11				
Genotypes	Genotype frequency		χ2	p	df
	observed	expected			
G /G	0,81	0,79	0,02		
G /C	0,17	0,19	0,26		
C /C	0,02	0,01	1,07		
Total	1	1	1,35	0,239	1

Table 3.
Frequency of occurrence of polymorphisms of the studied genes

Genotypes	Main group n = 96	Control group n = 83	χ^2	p	OR (95 % CI)
C174G polymorphism in the IL6 gene					
Genotype C/C	70,83 % (68)	79,52 % (66)	1,8	0,6	0,6 (0,31 - 1,25)
Genotype C/G	25% (24)	16,87% (14)	1,8	1,6	1,6 (0,79 - 3,42)
Genotype G/G	4,17% (4)	3,61% (3)	0,0	1,2	1,2 (0,25 - 5,33)

Note: n – number of people examined, χ^2 - chi-square, OR- odds ratio, 95% CI – 95% confidence interval OR, p – level of significance between groups.

When analyzing the data obtained, we found that in patients with diabetes mellitus, aggravated by the development of DFS, there were differences in the frequency of occurrence of the C174G polymorphisms of the IL6 gene. The C/G genotype of the indicated polymorphism of the IL6 gene was 1.4 times more common in patients of the main group with diabetic foot syndrome. The frequency of occurrence of the genotype G/G polymorphism C174G of the IL6 gene in patients with diabetic foot

syndrome was 1.2 times more common than in the control group.

It has also been established that in the presence of genotype C/C polymorphism C174G of the IL6 gene, the risk of developing diabetic foot syndrome is reduced by 50% (OR = 0.6 (Cl 0.31 - 1.25), which indicates its protective function in relation to the risk of diabetic foot syndrome in patients with diabetes mellitus.

SNP rs1800795 (-174G/C) of the IL-6 gene in T2DM was first described in 2003, showing that the GG phenotype is the genetic determination of inflammation in the development of T2DM [8,9,11]. Significant IL-6 174G/C gene sequence variation was widely distributed with varying frequencies in T2DM and healthy controls; this risk allele was also responsible for elevated serum IL-6 levels in genetically susceptible individuals [13]. Multiple studies have provided clear evidence that IL-6 single nucleotide polymorphism C-174G is a risk factor for type 2 diabetes and contributes to higher serum interleukin-6 levels among participants [4,6,12].

The impact of the C174G polymorphism in the IL6 gene may be exacerbated in combination with the presence of additional risk factors for the development of DFS, which may be caused by poor nutrition, medication use, bad habits or concomitant diseases. In order to effectively prevent DFS, it is advisable to recommend genotyping of the C174G polymorphism in the IL6 gene not separately, but in combination with other factors. This pathogenetic approach will make it possible to more reliably identify the genetic prerequisites for the risk of developing DFS.

Conclusion. Thus, the results obtained demonstrate that the presence of genotype C/G polymorphism C174G of the IL6 gene has a clear correlation with susceptibility to the development of diabetic foot syndrome. Carriage of the C/C genotype of the C174G polymorphism of the IL6 gene is associated with a protective effect regarding the development of diabetic foot syndrome. Thus, an integrated approach to the study of polymorphism of genes that influence the formation of diabetes mellitus will serve as the basis for the prevention and treatment of one of the catastrophic complications of diabetes mellitus.

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