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Genetic Predictors of Development of Diabetic Foot Syndrome in Patients with Diabetes Mellitus

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ABSTRACT: An increase in the population of patients with diabetes mellitus affects the incidence of diabetic foot syndrome (DFS), as its chronic complication. Among the various types of growth factors that play a role in the development of late complications of diabetes, vascular endothelial growth factor, VEGF, is currently in the focus of attention. The study conducted by the authors was aimed at determining the relationship between the G634C polymorphism of the VEGFA rs2010963 gene and the predisposition to the development of diabetic foot syndrome in patients with diabetes mellitus. We examined 96 patients aged 39 to 76 years with diabetes mellitus complicated by diabetic foot syndrome. Based on the studies, it was determined that the G634C polymorphism in the VEGFA gene (rs2010963) is involved in the formation and development of diabetic foot syndrome in patients with diabetes mellitus.

KEYWORDS: Diabetes mellitus, Diabetic foot, Genetic polymorphism, Vascular endothelial growth factor.

INTRODUCTION

Diabetes mellitus (DM) is a global health problem that affected about 411 million people in 2014 [1]. The incidence of DM has doubled since 1980, according to the World Health Organization [2]. An increase in the population of DM patients affects the incidence of diabetic foot syndrome (DFS) as a chronic complication of DM. The incidence of DFS tends to increase and becomes a significant burden in the healthcare system [3]. Up to 15–25% of diabetic patients will develop diabetes during their lifetime [4]. The prevalence of DFS in the diabetic population is 4–10% and is more common in older patients. It is estimated that about 5% of all patients with DM have a history of DFS, and the lifetime risk in patients with DM with this complication is 15% [4]. Without timely treatment, DFS can lead to amputation. This complication is one of the leading causes of disability and death in diabetic patients [4].

In recent years, the involvement of growth factors in the progression of DM and its complications has been reported. Among the various types of growth factors that play a role in the development of late complications of diabetes, vascular endothelial growth factor, VEGF, is currently in the focus of attention [5]. The role of VEGF is that it is a mitogen for vascular endothelial cells. It induces the process of collagenesis and angiogenesis, cleansing the matrix, facilitating the migration and germination of endothelial cells [1, 5]. Endothelial dysfunction as a mechanistic link in the pathogenesis of diabetic complications is partially mediated by VEGF. VEGF also induces collagenase, which promotes angiogenesis by clearing the matrix and facilitating endothelial cell migration and growth [12]. Hypoxia is one of the reasons for the increase in VEGF levels during wound healing. Oxidants produced in response to injury, such as hydrogen peroxide, and various other mediators produced at the wound site, such as epidermal growth factor, keratinocyte growth factor, altered growth factor, and tumor necrosis factor, also stimulate the production of VEGF by keratinocytes [13, 14]. The rs2010963 (G634C) polymorphism is located in the promoter region of the VEGF gene, which may have the ability to regulate gene expression levels. These SNPs have been investigated in a variety of conditions including diabetic ulcers and diabetic retinopathy [15].

It has been reported that VEGF gene polymorphisms affect the level of mRNA expression, so this polymorphism may be a potential marker when analyzing the role of genetics in complex diseases. It has been reported that there is a significant association between VEGF 2578* C/A polymorphism and susceptibility to DFS among the Iranian population [5]. Studies by Chinese scientists also reported that the rs6999947 polymorphism of the VEGF gene plays a significant role in the impact of DFS on the Han population [3]. Some evidence has shown that increased hypoxic conditions and impaired cellular response to hypoxia in diabetic patients are critical pathogenic factors in wound healing failure in DFU. Hyperglycemic conditions in patients with DM increase the production

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of mitochondrial reactive oxygen species (mtROS) and cause cellular hypoxia by downregulating aquaporin–1 (AQP1) expression. The hypoxic microenvironment will activate target genes that play a role in wound healing and remodeling, including the VEGF gene [7]. Polymorphic variants of the VEGFA gene encoding the production of vascular endothelial growth factor A, which is involved in the process of vascular wall remodeling, are associated with the development of diabetes mellitus, while there is no data on the association of this gene with the development of diabetic foot syndrome.

THE AIM OF THE RESEARCH

This research was aimed at determining the relationship between the G634C polymorphism of the VEGF rs2010963 gene and the predisposition to the development of diabetic foot syndrome in patients with diabetes mellitus.

THE MATERIALS AND METHODS OF THE RESEARCH

The diagnosis of DFS was established on the basis of the results of laboratory–instrumental (Doppler ultrasound) and molecular genetic studies. The study involved 96 patients aged 39 to 76 years with diabetes mellitus complicated by diabetic foot syndrome. The patients were hospitalized at the clinic of the Andijan Medical Institute. The control group consisted of 83 healthy individuals. Determination of allelic and genotypic variants of the VEGFA gene polymorphism (G634C) was carried out in the Department of Molecular Medicine and Cell Technology on the basis of 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 patients' peripheral blood lymphocytes using the AmpliPrime RIBO–prep isolation kit (Interlabservis, Russia). The study was conducted by quantitative real–time PCR analysis (Real–Time PCR). Amplification was performed using a thermal cycler for real–time PCR analysis – Rotor Gene Q, (Quagen, Germany). For the determination of genetic markers, test systems of the Sintol 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 criterion 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 obtained results, the application package "OpenEpi, 2009, VERSION 9.2.

RESEARCH RESULTS AND DISCUSSIONS

This study found that diabetic patients with DFS had a mean age of 56.5 ± 6.72 years in the range of 42–69 years, the average duration of DM disease was 8.62 ± 3.79 years in the age range of 5 to 20 years. Statistical analysis showed that there were no differences in age (p=0.683), duration of DM (p=0.415), sex (p=1.000), and occupation (p=0.761) between type 2 DM with and without DFS.

Table №1. Expected and observed frequencies of distribution of locus genotypes by RHV (polymorphism G634C in the VEGFA gene)

Main group					
Alleles	Allele frequency				
G	0,76				
C	0,24				
Genotypes	Genotypes frequency		$\chi 2$	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

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Control group					
Alleles	Allele frequency				
G	0,89				
C	0,11				
Genotypes	Genotypes frequency		χ^2	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

Groups	Ho	He	D*
Main group	0,32	0,37	–0,13
Control group	0,17	0,19	–0,13

Note: D = (Ho–He)/He

The allele frequencies of rs2010963G and rs2010963C in the main group of patients and the control group were 75.5% and 24.5% and 89.1% and 10.1%, respectively. At the same time, the distribution of alleles in the examined groups differed significantly; the unfavorable C allele was significantly higher among the main group of patients ($\chi2$=11.1; P=0.01; OR=2.7; 95% CI: 1.5 – 4.74) and in the subgroup of patients with purulent – necrotic complications DFS ($\chi2$=10.6; P=0.01; OR=2.8; 95% CI: 1.51 – 5.21). Allele C was shown to have a direct, statistically significant relationship with the disease, RR=1.2 (95% CI: 0.53 – 2.63). In the main group, compared with the population sample, a statistically significant decrease in the frequency of the G allele was shown, i.e. this allele has a protective effect $\chi2$=9.11; p=0.01; RR=0.8; 95% CI: 0.58 – 1.23). The homozygous G/G genotype had a protective effect in relation to the disease, since the chance of detecting this genotype was statistically significantly lower in a sample of patients compared to apparently healthy individuals (OR=0.3; 95% CI: 0.18 – 0.68; $\chi2$=9.5; p=0.01).

Table 2. Differences in the frequency of allelic and genotypic variants of the G634C polymorphism in the VEGFA gene in patient groups

Alleles and genotypes	Number of examined alleles and genotypes				$\chi2$	p	RR	95%CI	OR	95% CI
	Main group		Control group							
	n	%	n	%						
G	145	75,5	148	89,2	11,1	p = 0,01	0,8	0,58 – 1,23	0,4	0,21 – 0,67
C	47	24,5	18	10,8	11,1	p = 0,01	1,2	0,53 – 2,63	2,7	1,5 – 4,74
G /G	57	59,4	67	80,7	9,5	p = 0,01	0,7	0,45 – 1,21	0,3	0,18 – 0,68
G /C	31	32,3	14	16,9	5,6	p = 0,025	1,9	1,14 – 3,2	2,4	1,16 – 4,76
C /C	8	8,3	2	2,4	3,0	p = 0,1	3,5	1,77 – 6,76	3,7	0,83 – 16,25

The incidence of the heterozygous G/C genotype among patients in the main group is almost 2 times higher than its values in the control group (OR=2.4; 95% CI: 1.16–4.76; $\chi2$=5.6; p=0.025), homozygous C/C genotype - more than 2.7 times (OR=3.7; 95% CI: 0.83 – 16.25; $\chi2$=3.0; p=0.01).

VEGF is a mediator that regulates angiogenesis and is considered one of the main pro–angiogenic mediators in wound healing

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[11]. VEGF, which is normally expressed at low levels by epidermal keratinocytes, is upregulated in these cells in damaged skin. Keratinocytes can produce VEGF early in the wound healing process, but keratinocytes can also produce VEGF late in wound healing [12].

A significant association of VEGF polymorphisms with susceptibility to DFS has been reported in the Iranian population [3]. Several nucleotide polymorphisms (SNPs) have been identified in the VEGF gene.

In our study, the rs2010963 polymorphism of the VEGF gene was less common in patients with DFS than in the control group (59.4% versus 80.7%, respectively).

CONCLUSION

The G634C polymorphism in the VEGFA gene (rs2010963) is involved in the formation and development of diabetic foot syndrome in diabetic patients. The presence of the C allele and the G/C and C/C genotypes of the G634C polymorphism in the VEGFA gene significantly increases, and the carriage of the G allele and the G/G genotype reduces the risk of developing diabetic foot syndrome in patients with diabetes mellitus.

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