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PROGNOSTIC SIGNIFICANCE OF VEGFA AND IL6 GENE POLYMORPHISM IN DIABETIC FOOT SYNDROME

Dadabaev O. T.
Assistant of the Department of General Surgery

Aleinik V. A.
Professor of the Department of Normal and Pathological Physiology, MD

Musashaikhov Kh. T.
Professor of the Department of General Surgery, MD

Vasilevsky E.A.
Senior Lecturer of the Department of General Surgery
Andijan State Medical Institute

ANNOTATION
Diabetes mellitus is one of the serious medical and social problems of the modern world. Diabetic foot syndrome is one of the terrible complications of diabetes mellitus. In 55% of cases, this complication occurs in patients aged 25 to 75 years. To implement the basic principles of modern personalized medicine, it is relevant to study the genetic aspect of multifactorial diseases. The aim of the study was to analyze the correlation between the G634C polymorphisms of the VEGFA gene and the C174G polymorphisms of the IL6 gene and the development of diabetic foot syndrome in patients with diabetes mellitus. 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 G/G genotype of the G634C polymorphism of the VEGFA gene, as well as 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. Carrying the G/G genotype of the G634C polymorphism of the VEGFA gene and the C/C genotype of the C174G polymorphism of the IL6 gene is associated with a protective effect on the development of diabetic foot syndrome.

Keywords: diabetic foot, gene polymorphism, diabetes mellitus

АННОТАЦИЯ
Сахарный диабет является одной из серьезных медицинских и социальных проблем современного мира. Синдром диабетической стопы является одним из грозных осложнений сахарного диабета. В 55% случаев данное осложнение встречается у больных в возрасте от 25 до 75 лет. Для реализации основных принципов современной персонализированной медицины актуальным является исследование генетического аспекта мультифакториальных заболеваний. Целью исследования являлось проанализировать корреляционную связь полиморфизмов G634C ген VEGFA и C174G гена IL6 с развитием синдрома диабетической стопы у больных сахарным диабетом. В клиническую группу вошли 96 больных в возрасте от 40 до 75 лет с сахарным диабетом, осложненным синдромом диабетической стопы.

Контрольную группу составили 83 здоровых лиц. Полученные авторами результаты демонстрируют, что наличие генотипа G/G полиморфизма G634C гена VEGFA, а так же генотипа C/G полиморфизма C174G гена IL6 имеет четкую корреляционную связь с предрасположенностью к развитию синдрома диабетической стопы. Носительство генотипа G/G полиморфизма G634C гена VEGFA и генотипа C/C полиморфизма C174G гена IL6 ассоциировано с протективным эффектом относительно развития синдрома диабетической стопы.

Ключевые слова: диабетическая стопа, полиморфизм генов, сахарный диабет

ANNOTATSIYA

Qandli diabet zamonaviy dunyoning jiddiy tibbiy va ijtimoiy muammolaridan biridir. Diabetik oyoq sindromi diabetes mellitusning dahshatli asoratlaridan biridir. 55% hollarda bu asorat 25 yoshdan 75 yoshgacha bo'lgan bemorlarda uchraydi. Zamonaviy shaxsiylashtirilgan tibbiyotning asosiy tamoyillarini amalga oshirish uchun multifaktorial kasalliklarning genetik jihatini o'rganish dolzarbdir. Tadqiqotning maqsadi VEGFA genining G634C polimorfizmlari va IL6 genining C174G polimorfizmlari o'rtasidagi bog'liqlikni va diabetes mellitusli bemorlarda diabetik oyoq sindromining rivojlanishini tahlil qilish edi. Klinik guruh diabetik oyoq sindromi bilan asoratlangan qandli diabet bilan og'rigan 40 yoshdan 75 yoshgacha bo'lgan 96 nafar bemorni o'z ichiga oldi. Nazorat guruhi 83 nafar sog'lom odamdan iborat edi. Mualliflar tomonidan olingan natijalar VEGFA genining G634C polimorfizmining G/G genotipining mavjudligi, shuningdek, IL6 genining C174G polimorfizmining C/G genotipining mavjudligi IL6 geniga moyillik bilan aniq bog‘liqligini ko‘rsatadi. diabetik oyoq sindromining rivojlanishi. VEGFA genining G634C polimorfizmining G/G genotipini va IL6 genining C174G polimorfizmining C/C genotipini olib yurish diabetik oyoq sindromining rivojlanishiga himoya ta'siri bilan bog'liq.

Kalit so'zlar: diabetik oyoq, gen polimorfizmi, qandli diabet

The actuality. Diabetes mellitus is one of the serious medical and social problems of the modern world. Every year there is a steady increase in the incidence worldwide [1, 2, 13]. By 2030, the number of patients with this pathology is predicted to increase to 4.4% of the total population, which will affect more than 350 million people [2, 3, 4, 13]. Diabetic foot syndrome is one of the terrible complications of diabetes mellitus. In 55% of cases, this complication occurs in patients aged 25 to 75 years [2, 5, 6, 13]. In 80% of patients with diabetic foot syndrome, purulent-necrotic lesions of soft tissues develop, accompanied by a rapid and unpredictable course, often of a septic nature and in 30% of cases leading to high amputations [7,8, 18]. 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, 15, 16, 19, 20].

The aim of the research. In connection with the above, the aim of the study was to analyze the correlation between the G634C polymorphisms of the VEGFA gene and C174G of the IL6 gene with the development of diabetic foot syndrome in patients with diabetes mellitus.

Materials and Methods

Scientific work was carried out on the basis of the clinics of the Andijan State Medical Institute in the period from 2019 to 2022. The diagnosis of SDS was established on the basis of the results of laboratory-instrumental (USDG) and molecular genetic studies. The clinical group included 96 patients aged 40 to 75 years with diabetes mellitus complicated by diabetic foot syndrome. The patients were hospitalized. The inclusion criteria were: the presence of type 2 diabetes mellitus, the age of patients from 40 to 75 years. The control group consisted of 83 healthy individuals.

Determination of allelic and genotypic variants of polymorphism in the VEGFA gene (G634C) and in the IL6 gene (C174G) 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. Odds ratio (OR) and 95% confidence interval (CI) were calculated to establish the risk of developing DFS. For statistical processing of the obtained results, the application package "OpenEpi, 2009", VERSION 9.2 was used. Statistical analysis was based on the principles of the International Committee of Medical Journal Editors (ICMJE) and the Statistical Analysis and Methods in the Published Literature (SAMPL) guidelines. Nominal data were described with indication of absolute values, percentages.

The Results and their Discussion

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

The frequency of genotypes of the examined patients corresponded to the Hardy-Weinberg equilibrium, which allowed us to compare the carriage of these mutations in the studied groups. The results of the research are presented in tables 1, 2, 3.

Table 1. The frequency of occurrence of polymorphisms of the studied genes

Genotypes	Main group n = 96	Control group n = 83	χ^2	p	OR (95 % CI)
Polymorphism G634C in the VEGFA gene					
Genotype G /G	59,38 % (57)	80,72% (67)	9,5	0,01	0,3 (0,18 - 0,68)
Genotype G /C	32,29 % (21)	16,87% (14)	5,6	0,025	2,4 (1,16 - 4,76)
Genotype C /C	8,33 % (8)	2,41% (2)	3,0	0,1	3,7 (1,16 - 4,76)
Polymorphism C174G 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 examined, χ^2 - chi-square, OR- odds ratio (odds ratio), 95% CI - 95% confidence

interval OR, p - significance level between groups.

Table № 2 Expected and observed frequencies of distribution of locus genotypes by HWD (polymorphism G634C in the VEGFA gene)

Main group					
Alleles	Allele frequency				
G	0,76				
C	0,24				
Genotypes	Genotypes 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

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

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

As can be seen from our data, in the study of polymorphism P G634C of the VEGFA gene, the frequencies of alleles G and C 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 (χ2=11.1; P=0.01; OR=2.7; 95% CI: 1.5 - 4.74) and in the subgroup of patients with purulent-necrotic complications SDS (χ2=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 χ2=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; χ2=9.5; p=0.01).

Table № 3 Expected and observed frequencies of distribution of genotypes of the locus by HWD (C174G polymorphism in the IL6 gene)

Main group					
Alleles	Allele frequency				
C	0,83				
G	0,17				
Genotypes	Genotypes frequency		χ2	p	df
	observed	expected			
C /C	0,71	0,69	0,03		
C /G	0,25	0,28	0,27		
G /G	0,04	0,03	0,67		
Bcero	1	1	0,96	0,315	1

Control group					
Alleles	Allele frequency				
C	0,88				
G	0,12				
Genotypes	Genotypes frequency		χ2	p	df
	observed	expected			
C /C	0,8	0,77	0,05		
C /G	0,17	0,21	0,73		
G /G	0,04	0,01	2,67		
Bcero	1	1	3,46	0,064	1

Groups	Ho	He	D*
Main group	0,25	0,28	-0,1
Control group	0,17	0,21	-0,2

Note: D = (Ho - He)/He

When analyzing the obtained data, we found that in patients with diabetes mellitus aggravated by the development of DFS, there were differences in the frequency of occurrence of C174G polymorphisms of the IL6 gene. The C/G genotype of the indicated IL6 gene polymorphism was 1.4 times more common in patients of the main group with diabetic foot syndrome. The frequency of occurrence of the G/G genotype of the C174G polymorphism of the IL6 gene in patients with diabetic foot syndrome was 1.2 times more frequent than in the control group.

It has also been established that in the presence of the C/C genotype of the C174G polymorphism 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 developing diabetic foot syndrome in patients with diabetes mellitus[2,12, 14].

From the standpoint of metabolic disorders, the progression of type 2 diabetes mellitus is determined by a diverse combination of modifiable and non-modifiable risk factors. The main non-modifiable factors include age, gender, and genetic predisposition [11]. Carbohydrate metabolism disorders have a clear gender specificity and are more common in women, characterized by a more aggressive course [12]. A special role in this pathogenesis is played by gender-specific components of pathogenetic changes in carbohydrate metabolism, due to the action of sex hormones [11].

Thus, we found a correlation between the frequency of occurrence of the genotypes of the studied polymorphisms of the VEGFA and IL6 genes and the risk of developing diabetic foot syndrome in patients with diabetes mellitus[2,11,12, 14, 17].

Apparently, the studied polymorphisms in diabetes mellitus show their significance in the genesis of diabetic foot syndrome only in the presence of additional risk factors. The patterns established in the study can be used in the future to create mathematical multicomponent models for predicting the complications of diabetes mellitus.

Conclusion

Thus, the obtained results demonstrate that the presence of the G/G genotype of the G634C polymorphism of the VEGFA gene, as well as 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. Carrying the G/G genotype of the G634C polymorphism of the VEGFA gene and the C/C genotype of the C174G polymorphism of the IL6 gene is associated with a protective effect on the development of diabetic foot syndrome.

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