ORIGINAL ARTICLE

Association of Renin-angiotensin system Genes polymorphisms with Hypertension in patients with Type 2 Diabetes Mellitus

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ABSTRACT

Aim: To study the frequency distribution of alleles and genotypes and to identify the association of polymorphic markers *I/DACE* gene and *M235TAGT* gene with hypertension in patients with type 2diabetes mellitus (DM)in the Russian population.
Methods: 45 patients with type 2 DM and hypertension 2-3 stage were examined. The study of carbohydrate, lipid metabolism, morning albuminuria,24h-blood pressure monitoringweredone. Identification of polymorphic markers *I/DACE* gene and *M235TAGT* gene was performed using PCR-based assay.
Results: the evaluation of laboratory parameters in patients with type 2DM and hypertension revealed the excess of the target values of carbohydrate and lipid metabolism. When analyzing the frequency distribution of alleles and genotypes of the *ACE* gene, the ratio of pathological DD genotypes in the patient group in comparison with the control group was: 40.0% and 14.29% (p=0.043); II genotypes - 17.78% and 28.57% (p=0.453); heterozygotes (IDgenotypes) -42.22% and 57.14% (p=0.348); allele D was identified in 61.11% and 42.86%, respectively (p=0.096).The results of *AGT* gene genotyping showed that the frequency of TT genotype in patients with DM type 2 and hypertension was 37.78%, heterozygotes (MT) - 62.23%, and MM genotype - 0%. In the control group, the MT genotype was identified in all subjects (100%).
Conclusion: presence of the DD genotype of the *I/D*polymorphism *ACE* gene and the TT genotype of the *M235T* polymorphism *AGT* gene are associated with the development of hypertension in patients with type 2diabetes mellitus.
Keywords: Diabetes mellitus, hypertension, angiotensin-converting enzyme, angiotensinogen, gene, poly

INTRODUCTION

Diabetes mellitus (DM) is a global medical and social problem of our time. Data from numerous epidemiological studies indicate that in patients with DM the frequency of hypertension is 2 times higher than the general population values, amounting to 10-30% in type 1 DM and 60-80% in type 2 DM, with a combination of obesity - up to 93%.[1]

Currently, it is known that hypertension is a multifactorial, polygenic disease. The quantitative contribution of genetic factors to the development of hypertension is from 30 to 50%. Among the genes whose allelic polymorphism determines the increased risk of hypertension, much attention is paid to genes encoding components of the renin-angiotensin system (RAS).

It was found in patients with DM the RAS activity increases. Local renal RAS is of great importance in the development of systemic and intraglomerular hypertension. The mechanism of pathogenic effect of AT II in DM depends not only on vasoconstrictor action, but also mitogenic, proliferative, prooxidant and prothrombogenic activity and reduction of nitric oxide (NO). In addition, convincing data on the role of AT II in the development of insulin resistance (IR) and reduction of secretory activity of β-cells have been obtained.

The most studied RAS genes include the angiotensin-converting enzyme *(ACE)* gene and the angiotensinogen *(AGT)* gene.

The *ACE* gene is located in the long arm of the 17th chromosome at the 17q23 locus.About 20 polymorphic variants for the *ACE* geneare known, the most studied of which is polymorphism due to the insertion (presence) or action (absence) of the Alu repeat (insertion block of 287 nucleotide pairs) in the 16th intron.Increased *ACE* gene expression occurs during deletion of the Alu repeat (DD genotype). Currently, an association of this polymorphic marker with myocardial infarction has been identified in patients with type 1 and type 2 DM,[2] hypertension, left ventricular hypertrophy,[3,4] increased vascular stiffness,[5] and the development of diabetic nephropathy[6,7] and chronic kidney disease (CKD)[8] in different populations.However, these publications are quite contradictory.

The *AGT* gene is located on the long arm of the chromosome 1(1q42-q43).More than 3 dozens of polymorphic variants are described for it.One of the most studied is *M235T*. This is the nucleotide substitution of methionine for threonine at the 235th position of the amino acid sequence. According to the literature, in a number of populations an association of *TT* genotype with the development of hypertension,[9] including with diastolic, coronary heart disease (CHD), risk of myocardial infarction,[10] diabetic nephropathy and CKD in patients with DM type 2,[11,12] higher concentrations of ACE, AT I and AT II were found.[13]

However, often at present, data on the influence of genetic factors on the development of hypertension obtained by different researchers are quite contradictory. This is due, on the one hand, to the polygenicity and multifactorial nature of the pathogenetic mechanisms of hypertension formation, and, on the other hand, to population differences. The level of association of the same genetic markers in different populations can vary

significantly, which indicates the need for further study in this area.

The aim of the study was to study the frequency distribution of alleles and genotypes and to identify the association of *ACE I/D* gene and *AGT M235T* gene polymorphisms with hypertension in patients with type 2diabetes mellitus in the Russian population.

METHODS

The study was perfomed in accordance with the requirements of Good Clinical Practice and the WMA Declaration of Helsinki - Ethical principles for medical research involving human subjects. 45 patients with type 1 and type 2 DM and hypertension 2-3 stage were examined, including 27 women and 18 men. The average age is57.9±1.3 years, duration of diabetes 11.4±1.5 years, body mass index (BMI) 31.9±1.0 kg/m^2. Patients received diet therapy, hypoglycemic therapy, antihypertensive therapy.All patients underwent general clinical examination, anthropometric study included measurement of height, weight, calculation of body mass index (BMI). Laboratory studies included fasting glycemia, postprandial glycemia, glycated hemoglobin (HbAlc), lipid spectrum assessment - total cholesterol (TC), triglycerides (TG), low and high density lipoproteins (LDL and HDL). When genotyping, the control group consisted of 14subjects without disorders of carbohydrate metabolism and hypertension, comparable in age. A total of subjects were genotyped for *ACE* insertion(I)/deletion(D) and *AGT M235T* gene polymorphisms by polymerase chain reaction. Genomic DNA was isolated from the whole blood of patients using standard sets of primers ("Litech" - "SNP", Moscow).

Determination of albuminuria was carried out using a NycoCard apparatus in a single (morning) portion of urine. Glomerular filtration rate (GFR) was calculated by creatinine level according to the formula CKD-EPI-creat, 2009. 24h-blood pressure monitoring was carried out according to the standard method using the Valenta system apparatus (St. Petersburg).

Statistical analysis of the obtained data was carried out using the application package Statistica 10.0. The arithmetic mean (M) and the error of the arithmetic mean (m) were determined. The evaluation of the distribution of traits was carried out using the Shapiro-Wilk criterion; with the calculated value of $p>0.05$, the distribution was recognized as normal. The relative risk of disease in carriers of a certain allele and genotype was calculated as an indicator of the odds ratio (OR). Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

When assessing laboratory parameters in patients with DM type 2in combination with hypertension, an excess of the target values of carbohydrate, lipid metabolism was revealed. Fasting blood glucose level was 8.4±0.3 mmol/l, postprandial glycaemia - 9.5±0.2 mmol/l, and the level of HbA1c was 8.4±0.3%. Lipid profile of patients with type 2 DM and hypertension was atherogenic: TC - 6.2±0.2 mmol/l, LDL - 3.7±0.2 mmol/l, TG-2.2±0.3 mmol/l.GFR according to the formula CKD-EPI-creat, 2009 - 69.8±4.4 ml/min. The average level of albuminuria in the patient group was 17.3 mg/l, an indicator of more than 20 mg/lwas detected in 9 patients (20.0%)

When analyzing the results of 24h-blood pressure monitoring the average values of systolic blood pressure (BP) and diastolic BP in the daytime and at nightin patients on the background of antihypertensive therapy within normal values.However, the time index of diastolic BP significantly exceeded normal values and amounted to diastolic BP day - 73.9±7.3%, diastolic BP night - 87.3±7.1%, diastolic BP 24h - 75.1±7.0%.

Diabetic retinopathy of I and II stages (by Koner E., Porta M., 1992) was diagnosed in 10 patients (22.22%), diabetic nephropathy in the stage of microalbuminuria - in 9 patients (20.0%), macroangiopathies(CHD, cerebrovascular disease, atherosclerosis of the arteries of the lower extremities) - in 15 patients (33.3%).

ACE gene *I/D*polymorphism was analyzed by polymerase chain reaction in 45 hypertension diabetic cases and 14 healthy controls.The frequency distribution of genotypes and *I/D* allelic polymorphism of *ACE* gene in patients with type 2 DM and hypertension is presented in table 1.

According to published data in a healthy population, the carriage of the mutant D allele of the *ACE* gene in the homozygous state (DD genotype) is up to 20%.These data are comparable with the results obtained in this study: in the Russian population, DD genotype was detected in 14.29% of cases in the control group.While in the group of patients, carriage of DD genotype was 40.0%($p=0.043$).Specifically, for the *ACEI/D* polymorphism, our results showed a significant difference between hypertensive diabetic and normotensive groups across genotypes, although the OR for the DD genotype was not significant (table 1).

When analyzing the parameters of 24h-ambulatory blood pressure (BP) monitoring in carriers of D and I alleles of the *ACE* gene receiving antihypertensive therapy, no significant differences were demonstrated in the parameters of systolic and diastolic BP, time index, speed and magnitude of the early morning PBsurge.However, in patients with the DD genotype, there was a more significant violation of the BP circadian rhythm in the form of an insufficient decrease in diastolic BP at night: non-dipper profile in 15 patients (83.33±9.04%), dipper in 3patients (16.67±9.04%); in group with the II genotype – in 3 patients (37.50±18.3%),$p=0.035$,and in 5 patients (62.5±18.3%), $p=0.035$, respectively.

The frequency distribution of genotypes and *M235T* allelic polymorphism of *AGT* gene in patients with DM type 2 and hypertension is presented in table 2.According to published data in European populations, the frequency of mutations *M235T* (TT homozygotes) is from 15 to 20%, which is 1.5-2 times less than in the group of patients with DMtype 2and hypertension examined by us.Studies of the Polish and Lebanese populationsshowed a significant predominance of the TT genotype and the T allele in the group of patients with hypertension compared with the healthy control.[13]Particularlysignificant association was observed in patients with an unfavorable history of hypertension.[14]

According to a meta-analysis by Sethi A.A et al., an association of *M235T* polymorphism of the *AGT* gene with a risk of hypertension in Caucasians and indigenous people of Asia was revealed. Also, representatives of the Caucasoid race showed an increase of the of ATII plasma concentration by 5% in MT heterozygotes and by 11% in TT homozygotes compared with MM homozygotes.[15]

In our study, in homo- and heterozygotes for the T allele of the *AGT* gene, there were no differences in the levels of 24h-blood pressure monitoring. However, in patients with TT genotype, there was a tendency to increase the variability of systolic BP and diastolic BPduring the day and night and the non-dipper profile predominated: in 11patients (64.71%) compared to9 patients(32.14%) in MT homozygotes, p = 0.035.

Table 1 - Genotype/allele frequency comparison of the *I/DACE* gene among hypertensive patients with diabetes mellitus type 2 and normoglycemic normotensive subjects

Allele and Genotype	Frequency		t	p	Oddsratio	
	DM +Hyper-tension (n=45)	Control (n = 14)			OR	95% CI
AlleleI, %	38.89±5.14	57.14±9.52	1.69	0.098	0.318	0.123-0.822
Allele D, %	61.11±5.14	42.86±9.52	1.69	0.096	2.095	0.887-4.952
Genotype II, %	17.78±5.7	28.57±12.53	0.78	0.453	0.541	0.135-2.167
GenotypeID, %	42.22±7.36	57.14±13.73	0.96	0.348	0.548	0.163-1.843
Genotype DD, %	40.0±7.3	14.29±9.71	2.12	**0.043**	4.320	0.859-21.719

Table 2- Genotype/allele frequency comparison of the *M235TAGT*gene among hypertensive patients with type 2diabetes mellitus and normoglycemic normotensive subjects

Allele and Genotype	Frequency		t	p	Oddsratio	
	DM +Hyper-tension(n = 45)	Control (n = 14)			OR	95% CI
AlleleT, %	68.89±4.88	50.0±9.62	1.75	0.084	2.214	0.932-5.258
Allele M, %	31.11±4.88	50.0±9.62	1.75	0.084	0.452	0.190-1.072
Genotype TT, %	37.78±7.23	0	5.23	**0,000015**	-	-
Genotype MT, %	62.23±7.23	100	5.23	**0,000006**	-	-
Genotype MM, %	0	0	-	-	-	-

CONCLUSION

Thus, for the *ACEI/D* polymorphismand *AGT M235T* polymorphism, our results showed a significant difference between hypertensive patients with type 2diabetes mellitus and normotensive groups across genotypes.The presence of DD and TT genotypes of these markers are associated with the development of hypertension in patients with type 2diabetes mellitus.

Declaration of author's competing interests: The authors declare no conflict of interest.

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