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Obesity and functional state of kidney depends on VEGF polymorphism

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Abstract.

The aim this study was to investigate the relationship of polymorphism VEGF gene to the functional state of kidneys in patients with various phenotypes of obesity. We examined 240 persons (mean age of 43.4±7.3), which were distributed in several groups. The first group included 90 patients with obesity with metabolic disorders. The second group was presented by 50 patients - they do not display signs of metabolic disorder. The control group was composed of healthy. We detected high levels of VEGF in blood and urine, A2, type IV collagen and predominance of minor C allele in group 1. In group 2, there were VEGF increase in blood and urine (at optimal values of GFR and A1). We detected a link between polymorphism of promoter region of -634 G/C with levels of SBP, GFR, A2, type IV collagen and increased expression of VEGF in blood and urine. The increase in VEGF in the urine reflects the activation of angiogenesis and endothelial dysfunction, aggravated during the development and progression of chronic kidney disease. In the group with obesity and hypertension, we detected an increase of VEGF level in urine, while maintaining the normal range of A and GFR. The distribution of genotypes in the region -634 G/C of VEGF gene revealed the associating carriage of the minor allele in the group 2. In the group 1, albuminuria 2 was associated with the CC variant of VEGF gene (-634 G/C).

Key words: obesity, gene, polymorphism, VEGF, kidney, metabolic disorder.

INTRODUCTION

According to the Russian national guidelines for management of patients with obesity, the prevalence of overweight and obesity constitute 59.2% and 24.1%, respectively [1]. However, 10-40% of patients with obesity do not display violations corresponding to generally accepted criteria of metabolic illness: dyslipidemia, disorders of carbohydrate metabolism, insulin resistance and high blood pressure (BP). This variant of obesity is currently designated as a special phenotype of the metabolically healthy obesity. It has been shown that metabolic abnormalities are more significant determinants in the development of diabetes mellitus type 2 (DM-2) than the presence of obesity [2]. It is known that obesity, even in the absence of violations of carbohydrate metabolism, is an independent risk factor for chronic kidney disease (CKD), which in turn is a risk factor for adverse cardiovascular outcomes [3, 4].

Vascular endothelial growth factor (VEGF) belongs to the family of proteins stimulating the growth and proliferation of endothelial cells of blood vessels [5]. In the glomerulus, VEGF promotes the maturation of the podocytes, mesangial cells and the formation of functional glomeruli in children; it is also necessary for the functioning of the glomeruli in adults [6]. The increase of VEGF level in patients with abdominal obesity, the relationship with lipid metabolism and inflammation factors are proven facts. There is also evidence of the relationship between the level of urinary excretion of VEGF and albuminuria (A) in patients with arterial hypertension (AH) and type 2 diabetes [7, 8]. Recently scientists established a relationship between the functional polymorphism of VEGF gene with the level of its spontaneous production, especially during the combination of obesity and diabetes mellitus type 2 [9-11]. However, in the literature there are no data evaluating the contribution of VEGF gene polymorphism in the development of certain phenotypes of obesity and functional state of the kidneys. In this connection, the aim this study was to investigate the relationship of polymorphism VEGF gene to the functional state of kidneys in patients with various phenotypes of obesity.

MATERIALS AND METHODS

We examined 140 persons aged 25 to 55 years, (M=43.4±7.3), which were distributed in several groups. The first group included 90 patients (women among them - 61%) with obesity (according to WHO criteria) with metabolic disorders (IDF criteria, 2005) – we marked them as metabolically unhealthy obesity (MUO). The second group was presented by 50 patients (women among them - 50%) – we marked this group as metabolically healthy obesity (MHO) – they do not display signs of metabolic disorder according to criteria of WHO and IDF. The control group was composed of healthy persons (n=50, women - 50 %) with normal body mass index (BMI). Exclusion criteria from the study were secondary forms of hypertension, stage III hypertension, coronary heart disease, diabetes, previously established chronic kidney disease, and kidney stones. These patients do not use a regular antihypertensive therapy and statins.

All patients underwent to clinical and laboratory examination, glomerular filtration rate (GFR) was calculated with the formula CKD-EPI ml/min/1.73 m^2. Degree of reduced GFR and albuminuria level (AL) were evaluated in accordance with the recommendations by KDIGO 2017 [12]. The concentration of VEGF in the serum and morning urine of the examined individuals were determined by ELISA using a set of reagents "VEGFA – ELISA – BEST" (JSC "Vector-Best", Novosibirsk, Russian Federation). The level of albuminuria and type IV collagen in the morning portion of urine of patients was determined by ELISA using reagents "ELISA Micro-Albumin" (Orgentec, Germany) and "Argutus Medical Collagen IV EIA" (Daiichi Fine Chemical Co., Ltd., Japan). To identify single-nucleotide polymorphisms (SNP) of VEGF gene in the region -634G, we used method of real-time PCR. Total DNA was extracted from venous blood samples using the kit "DNA-Sorb-B" (JSC InterLabService, Moscow, Russian Federation). VEGF gene polymorphism (-634G/C) was performed with thermocycler "CFX-96" ("Bio-Rad Laboratories, Inc.", USA) using allele-specific PCR "SNP Screen" (CJSC "Synthol", Moscow, Russian Federation) and detection of products in real time regime.

For statistical data processing we used "Statistica 10.0" software. Evaluating data with a normal distribution, we used the average value (M) and standard deviation (SD); student's *t*-test. For multiple comparisons between groups we used the *h*-criterion of Kruskal-Wallis. Paired comparisons in the same module were performed using the Mann-Whitney test. Relationship of characteristics were evaluated using regression analysis with determination of Spearman's rank correlation. To describe the ratio of the frequencies of genotypes and alleles of the studied genes, we used the method of χ^2. The differences in the two

populations were calculated by odds ratio (OR), which was determined using the "case-control" approach for various models of inheritance. OR ≥1.0 probability of events was evaluated as high. The correlations of the studied qualitative characteristics to assess the association of polymorphism of the studied gene with a high/low level markers of the functional state of the kidneys and markers of kidney damage were performed on the contingency table (a cross tabulation). Quantitative traits were transformed to qualitative using quartile deviations to evaluate the dependence between a factor and genotype. Relationship strength of variables was measured by Pearson coefficient of contingency. Dependence were considered statistically significant at significance level $p< 0.05$.

RESULTS

All patients from the MUO group of we detected I-II stage hypertension according to European guidelines for hypertension; the average duration of hypertension was 4.1±2.5 years. Main indicators of the groups are presented in Table 1.

Table 1. Clinical parameters in patients under study (Mean±m)

Parameter	Group 1 (MUO) (n = 90)	Group 2 (MHO) (n = 50)	Control (group 3) (n = 50)	*p* Kruskal-Wallis H-test (2,N=190) Mann-Whitney U-test
Systolic blood pressure, mm Hg	152±7,1	125±7,4	125±5,1	p=0,01 $p_{1-2;1-3}$ = 0,001 p_{2-3} = 0,8
Diastolic blood pressure, mm Hg	105±7,2	74±8,5	75±4,3	p=0,01 $p_{1-2;1-3}$ = 0,001 p_{2-3} = 0,8
Glycemia, mmol/l	5,8±0,5	4,9±0,7	4,2±0,5	p=0,1 p_{1-2}= 0,04 p_{2-3}= 0,2
Postprandial glucose, mmol/l	5,9±1,2	5,1±1,1	4,8±1,0	p=0,1 p_{1-2}=0,04 p_{1-3}=0,02 p_{2-3}=0,03
Triglycerides, mmol/l	2,7±0,5	1,2±0,2	1,2±0,5	p=0,03 $p_{1-2;1-3}$ = 0,03 p_{2-3}= 0,05
High density lipoproteins, mmol/l	1,1±0,3	1,4±0,2	1,4±0,1	p=0,9
Very low density lipoproteins, mmol/l	4,0±0,6	2,7±0,3	2,4±0,4	p=0,02 $p_{1-2;1-3}$ = 0,02 p_{2-3} = 0,06
VEGF, pg/ml (blood)	334,0±70,8	172,0±82	78,5 ± 15,3	p=0,01 p_{1-2}= 0,01 $p_{1-3;2-3}$ = 0,001
VEGF, pg/ml (urine)	70,2 ± 16,3	42,3 ± 12,1	15,8±7,6	p=0,01 p_{1-2}= 0,01 $p_{1-3;2-3}$ = 0,001
A, mg/ml	28,6± 3,1	14,6±4	9,4 ±2,5	p=0,02 $p_{1-2;1-3}$= 0,01 p_{2-3}= 0,1
Type IV collagen, μg/mmol of creatinine	3,9±1,4	0,3±0,1	0,2±0,02	p=0,02 $p_{1-2;1-3}$= 0,01 p_{2-3}= 0,04

Table 2. Biomarkers depending on the functional state of kidney.

Biomarker	Mean±m Kruskal-Wallis H-test (2,N=127) Mann-Whitney U-test			
	GFR >90<125 ml/min/1.73m^2 (n = 88)	GFR <90>60 ml/min/1.73m^2 (n = 36)	GFR <60 >45 ml/min/1.73m^2 (n = 3)	p
A, mg/ml	21,0±7,1	33,2± 3,1	38,7± 2,5	p=0,04 p *=0,02 p**=0,01 p#= н.д.
Type IV collagen, μg/mmol of creatinine	3,0±2,3	4,3±1,3	11,0±4,0	p=0,04 p *=0,02 p**=0,01 p#= н.д.
VEGF, pg/ml (blood)	258,0±100,5	378,0±135,3	541,0±134,4	p=0,02 p *=0,01 p**=0,01 p#= н.д.
VEGF, pg/ml (urine)	49,0±12,1	85,9 ±11,0	93,8±15,2	p=0,04 p *=0,02 p**=0,01 p#= н.д.

Table 3. Frequency distribution of alleles and genotypes of the polymorphic marker rs 2010963 of the VEGF gene in groups (multiplicative and general inheritance model, χ^2-test, df = 1).

Alleles and genotypes	Frequency of alleles and genotypes		χ^2	p	*OR*	
	Group 1 (MUO)	Group 3 (control)				
	n=90	n=50			Value	CI 95%
G	80/0,442	60/0,600	4,8	0,028	0,53	0,3 – 0,94
C	100/0,558	40/0,400			1,89	1,07 – 3,35
G/G	16/0,173	19/,378	5,45	0,066	0,34	0,13 – 0,88
G/C	48/0,538	22/0,444			1,46	0,65 – 3,25
C/C	26/0,288	9/0,178			1,88	0,71 – 4,95
Alleles and genotypes	**Group 2 (MHO) (n=50)**	**Group 3 (control)**	χ^2	p	*OR*	
					Value	CI 95%
G	60/0,597	60/0,600	0,00	0,968	0,99	0,51 – 1,91
C	40/0,403	40/0,400			1,01	0,52 – 1,96
G/G	18/0,355	19/,378	0,12	0,943	0,91	0,35 – 2,34
G/C	22/0,484	22/0,444			1,17	0,47 – 2,93
C/C	8/0,161	9/0,178			0,89	0,26 – 3,03
Alleles and genotypes	**Group 1 (n=90)**	**Group 2 (n=50)**	χ^2	p	*OR*	
					Value	CI 95%
G	80/0,442	60/0,597	3,71	0,04	0,54	0,28 – 1,01
C	100/0,558	40/0,403			1,87	0,99 – 3,53
G/G	16/0,173	18/0,355	4,08	0,130	0,38	0,14 – 1,06
G/C	48/0,538	22/0,484			1,24	0,51 – 3,03
C/C	26/0,288	8/0,161			2,11	0,68 – 6,52

Table 4. The frequency of allelic variants of the VEGFA gene (-634 G/C), depending on the level of microalbuminuria

Factor/Genotype/Allele		A1(n=118)%±m	A2 (n=22)%±m	QR (95%Cl)	p
VEGF (-634 G/C)	GG,%	28,5±7,6	14,7±4,3	2,3(0,8–6,2)	0,12
	GC,%	62,8±8,1	55,8±6,0	1,3(0,5 –3,0)	0,49
	CC,%	8,5±4,7	29,4±5,5	1,0 (0,06–0,8)	0,01
Allele	G-allele,%	60,0±5,8	42,6±4,2	2,0 (1,1–3,6)	0,018
	C- allele,%	40,0±5,8	57,3±4,2	1,0 (0,2–0,8)	0,04

In group 1 (MUO), the optimal GFR (>90 ml/min/1.73m^2) was established in 58.8% (n=53) of patients. Other persons - 28.8% (n=26) showed a slight decrease (>60<90 ml/min/1.73m^2), 8.8% of patients (n=8) had an increase in GFR (>125 ml/min/1.73m^2), in 3.3% (n=3) we revealed a moderate decrease in GFR (>45 to<60 ml/min/1.73m^2). In group 2 the optimal GFR was found in 70% (n=35) of patients; 20% (n=10) showed a slight decrease, 10% (n=5) of patients had an increase in GFR. Level 2 of albuminuria (A2) was detected in 24.4 % of patients (n=22) of the 1st group, while in the 2nd group (MHO), such patients were absent. In the MUO group, the values of A2, VEGF in the blood and urine, and collagen type IV were significantly higher than in the MHO group and in the control one. It was found that GFR decline results in increases in concentration of biomarkers: A,VEGF and collagen type IV in the urine (Table 2).

In the group of MUO, there was a positive correlation between A value and the level of diastolic blood pressure (DBP) (r = 0.7; p = 0.02), total cholesterol (r = 0.41; p = 0.04), VEGF in blood (r = 0.36; p = 0.03) and urine (r = 0.47; p = 0.03), collagen type IV (r = 0.52; p = 0.01) and negative correlation with a value of GFR (r = -0.5; p = 0.03). It was also established an association between VEGF in the urine and the level of systolic blood pressure (SBP) (r = 0.49; p = 0.03), type IV collagen (r = 0.34; p = 0.02), and uric acid (r = 0.49; p = 0.04). The negative correlation was found with GFR (r = -0.46; p = 0.03). There was a direct correlation of type IV collagen with the level of VEGF in the blood (r = 0.43; p = 0.02) and reverse one with GFR (r = -0.46; p = 0.03).

In the study of combinations of allelic variants of -634G/C polymorphism in VEGF gene in the group of MUO patients, we revealed a predominance of the minor allele - in 55.7% (p = 0.028; QR = 1.89) in contrast to the control group and MHO (40%). Between the MHO group and the control one we did not detect significant differences in the frequency of occurrence of genotypes and alleles (Table 3).

To examine the relationship of VEGFA gene polymorphism (-634 G/C) to the functional state of the kidneys, our patients were divided into two groups according to the albuminuria level: 118 patients with the A1 level, and 22 patients with A2 level. Major G allele of polymorphism -634 G/C of VEGFA gene significantly more often detected in patients with A1 - 60% (χ^2 = 5.5; p = 0.018; QR = 2.0), while minor allele, on the contrary, was significantly more prevalent in patients with A2 - in 57.3% (χ^2 = 5.5; p = 0.01; QR=1,0). Adverse homozygote CC was significantly more often in the group with A2 level - in 29.4% (χ^2 = 6.9; p = 0.01; QR = 1,0) (Table 4). We found a link between the polymorphism of promoter region of -634 G/C gene with the SAP level (p = 0.014), GFR (p = 0.014), A2 (p = 0.031), type IV collagen (p = 0.032) as well as with increased expression of VEGF in the blood (p<0.001) and urine (p=0.005).

DISCUSSION

Results of the study confirmed that obesity, especially in combination with hypertension, is a risk factor for loss of kidney function [1, 3]. We found a high VEGF level in blood and in urine in the groups of obesity in comparison with the control, which can be explained by several factors. Obesity contributes to hypoxia of

adipocytes in connection with the inability of the vasculature to increase following rapid growth of adipose tissue, which stimulates the increased expression of angiogenic factors, including VEGF [13]. In the MUO group, the levels of VEGF and type IV collagen were significantly higher than in the comparison groups, which confirms the multiplication of adverse effects on the kidney when the combination of obesity and hypertension takes a place.

It was reported before that the degree of decrease in renal function is associated with increased concentrations of markers of renal damage and of type IV collagen in the urine [4, 14]. In our study, the A2 was detected in the MUO group, however, in the group of MHO the A values were in the range of higher normal values (up to 20 mg/ml). VEGF is a critical factor in the regulation of glomerular barrier function in health and disease; its production contributes to the development of atherosclerotic process and progression of chronic kidney disease [8]. The increase in type IV collagen also favors the activation of sclerotic processes within the glomerulus [4]. The obtained relationship of VEGF level with excretion of type IV collagen, as well as VEGF correlation with GFR and A favors the activation of sclerotic processes within the glomerulus. The increase in VEGF in the urine in the MHO group with a normal A level and optimal GFR, is apparently caused by the activation of angiogenesis.

Analysis of distribution of allele polymorphism in VEGF gene (-634 G/C) did not reveal significant differences between the MHO group and the control group, while the prevalence of carriers of the minor allele was associated with the MUO. Association of promoter region polymorphism -634 G/C gene with the level of GFR and A, type IV collagen in the urine, as well as a significant frequency of pathological CC allele in the group with A2, could indicate the contribution of this polymorphism in predisposition to CKD: it was confirmed by the data of other researchers [14]. The identified association of a polymorphism in the promoter region of the -634 G/C gene with increased expression of VEGF in the blood and urine, may suggest on the contribution to the activation of angiogenesis in the carrier groups. However, some other mechanisms may be involved like mentioned previously [15, 16].

CONCLUSIONS

The increase in VEGF in the urine reflects the activation of angiogenesis and endothelial dysfunction, aggravated during the development and progression of chronic kidney disease. In the group with obesity and hypertension, we detected an increase of VEGF level in urine, while maintaining the normal range of A and GFR. The distribution of genotypes in the region -634 G/C of VEGF gene revealed the associating carriage of the minor allele in the MUO group. In the MUO group, albuminuria 2 was associated with the CC variant of VEGF gene (-634 G/C).

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