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Abstract **УДК 616.12-008.331.1:613.25:616.43-008.6:575.22**

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PARTICULARITIES OF METABOLIC INDICATORS IN PATIENTS WITH ARTERIAL HYPERTENSION AND CONCOMITANT OBESITY DEPENDING ON ADIPONECTIN GENE POLYMORPHISM

Relevance. The main component of the metabolic syndrome (MS) is the abdominal obesity (AO) which inevitably leads to insulin resistance (IR). Adiponectin (AN) secreted by the adipocytes protects from the IR development, increases the sensitivity of skeletal muscles to insulin, reduces intake of free fatty acids in a liver and, thus reduces the synthesis of atherogenic lipoproteins. Some investigations prove that the level of the adiponectin in blood plasma correlates back with the mass of the fatty tissue, the waist-hip ratio and IR expressiveness. With obesity, AN secretion decreases, losing its protective role as for the risk of IR development and metabolic violations.

It is known that ADIPOQ gene has several polymorphic sites, which influence the production and activity of AN. Allelic options of its polymorphic marker G276T are associated with the development of the AO, IR, and diabetes.

The aim of the study was to investigate the association of the genetic polymorphism of the G276T marker of the adiponectin gene with changes in metabolic parameters in patients with arterial hypertension (AH) and concomitant obesity.

We examined 300 patients with AH 45 to 55 years old who gave informed written consent to participate in the study and met the inclusion criteria. Group 1 consisted of 200 patients with AH and class I–II obesity, group 2 – 50 patients with AH and normal body weight, group 3 – 50 patients with AH and overweight. The control group consisted of 30 practically healthy individuals, in whom AH and obesity were excluded on the basis of clinical and instrumental examination data.

As a result of the study the association of T-allele of the polymorphic marker G276T of the adiponectin gene with the development of comorbidity of AH and obesity was established. It has been proven that adiponectin gene polymorphism influenced changes in metabolic parameters in hypertensive patients with obesity: more pronounced impairment of metabolic parameters in G/T and T/T genotypes as compared to G/G genotype. G/T and T/T genotypes of the polymorphic marker G276T of the adiponectin gene were associated with a significantly higher body mass index, higher triglyceride levels, more pronounced insulin resistance and adipokine imbalance.

Keywords: arterial hypertension, obesity, adiponectin gene polymorphism, insulin resistance, metabolic parameters.

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Резюме

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ОСОБЛИВОСТІ МЕТАБОЛІЧНИХ ПОКАЗНИКІВ У ПАЦІЄНТІВ ІЗ ГІПЕРТОНІЧНОЮ ХВОРОБОЮ І СУПУТНІМ ОЖИРІННЯМ ЗАЛЕЖНО ВІД ПОЛІМОРФІЗМУ ГЕНА АДИПОНЕКТИНУ

Мета дослідження полягала у вивченні асоціації генетичного поліморфізму маркера G276T гена адипонектину зі змінами метаболічних показників у пацієнтів із гіпертонічною хворобою (ГХ) і супутнім ожирінням.

Обстежено 300 пацієнтів із ГХ віком від 45 до 55 років, які дали інформовану письмову згоду на участь у дослідженні і відповідали критеріям включення. До першої групи ввійшло 200 пацієнтів із ГХ у поєднанні з ожирінням I–II ступенів, до другої групи – 50 пацієнтів із ГХ і нормальною масою тіла, до третьої групи – 50 пацієнтів із ГХ та надлишковою масою тіла. Контрольну групу становили30 практично здорових осіб, в яких ГХ та ожиріння були виключені на підставі даних клініко-інструментального дослідження.

У результаті проведеного дослідження встановлена асоціація алеля Т поліморфного маркера G276T гена адипонектину з розвитком коморбідності ГХ та ожиріння. Доведено, що поліморфізм гена адипонектину впливав на зміни метаболічних показників у пацієнтів із ГХ та ожирінням: при G/T- і T/T-генотипах мали місце більш виражені порушення метаболічних показників, ніж при G/G-генотипі. G/T- і T/T-генотипи поліморфного маркера G276T гена адипонектину були пов'язані з достовірно більшим індексом маси тіла, вищими рівнями тригліцеридів, більш вираженою інсулінорезистентністю та дисбалансом адипокінів.

Ключові слова: гіпертонічна хвороба, ожиріння, поліморфізм гена адипонектину, інсулінорезистентність, метаболічні показники.

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Вступ

For decades, essential hypertension (EH) and obesity are considered to be the most common non-infectious diseases in the world, in the development of which a significant role belongs to a combination of hereditary and acquired factors [1–5].

One of the mechanisms of development of insulin-resistant (IR) states is a dysfunction of the expression of adipose tissue hormones, in particular adiponectin [2, 6, 9, 10].

Adiponectin is a hormone that is synthesized and secreted by white adipose tissue (predominantly in the visceral adipocytes). It is known that adiponectin inhibits the differentiation of preadipocytes, which confirms its possible effect on the regulation of adipose tissue. In addition, adiponectin is involved in the regulation of energy homeostasis, and has anti-inflammatory and anti-atherogenic effects. In obesity there is a decrease of activity and concentration of adiponectin in the blood (unlike other adipokines, including leptin, resistin, and tumor necrosis factor-α, activity of which increases). The results of several studies have shown that the level of adiponectin in blood plasma inversely correlates with the mass of adipose tissue, the ratio of waist circumference to hip circumference and the IR severity. It is believed that adiponectin protects against hyperglycemia, IR and atherosclerosis [7–9, 11, 12].

Adiponectin production and activity depend on the structure of the adiponectin gene (ADIPOQ) localized on chromosome 3 at locus 3q27. In the presence of several polymorphic sites in the ADIPOQ gene, polymorphic marker G276T is considered to be the most clinically significant in the development of obesity, IR and type 2 diabetes [7–9, 13–16].

Thus, the study of polymorphism of candidate genes of lipid and carbohydrate metabolism is relevant, because, on one hand, it will contribute to the allocation of risk groups for certain diseases, and on the other hand, it will help in the future in

the prescription of drugs that more specifically act on specific cellular mechanisms of formation of pathological processes.

The objective of the research was to study the association of genetic polymorphism of ADIPOQ gene marker G276T with changes in metabolic parameters in patients with EH and concomitant obesity.

Clinical characteristics of patients and research methods. 300 patients with EH aged 45 to 55 years, who gave informed written consent to participate in the study and met the inclusion criteria, were examined. The first group consisted of 200 patients with EH in combination with class I–II obesity, the second group consisted of 50 patients with EH and normal body weight, the third group consisted of 50 patients with EH and excessive weight. The control group consisted of 30 healthy individuals in whom EH and obesity were excluded on the basis of clinical and instrumental examination data.

Criteria for inclusion in the study: EH II stage, 2nd degree; class I obesity (BMI – 30–34.9), class II obesity (BMI – 35–39.9), abdominal obesity (according to IDF criteria, 2005 [17]): waist circumference > 94 cm for men and > 80 cm for women; chronic heart failure (CHF) I–II functional classes (FC); preserved ejection fraction (EF) of the left ventricle (LV); normal glomerular filtration rate (GFR), normokreatininemia, absence of proteinuria (only microalbuminuria is allowed); age of patients – 45–55 years.

Criteria for exclusion from the study: the presence of comorbidities in patients with EH (acute coronary syndrome, post-infarction cardiosclerosis, severe rhythm and conduction disorders, rheumatic heart disease, systemic connective tissue diseases, cancer, symptomatic hypertension, thyroid disease, acute inflammatory processes); stage III, grade 3 EH; grade III obesity; type 1 and type 2 diabetes; CHF III–IV FC; moderately reduced and reduced LV EF; reduced GFR, the presence of proteinuria; age of patients less than 45 and more than 55 years; refusal of patients to participate in the study.

Physical examination of patients included measurement of height, body weight and calculation of BMI, kg/m2:

BMI = body mass (kg)/height (m^2).

Also, waist circumference (WC), hip circumference (HC) and the waist/hip index (WHI), calculated as a ratio of WC to HC, were measured in patients.

Fasting venous blood glucose, insulin, total cholesterol (TC), triglycerides, high density lipoprotein cholesterol (HDL cholesterol) and low density lipoprotein cholesterol were determined by standard biochemical methods.

IR was calculated using the HOMA model:

HOMA-IR = blood glucose (mmol/l) × blood insulin (μAU/l) /22.5.

Functional state of adipose tissue was assessed using blood leptin and adiponectin levels. Leptin was determined in serum using "Leptin ELISA" kits (DRG Diagnostics, Germany). Adiponectin levels were determined using the test-system "Avi Bion Human Adiponectin (Acrp30) Elisa Kit" (Ani Biotech Oy Orgenium Laboratories business Unit, Finland).

G276T genetic polymorphism was established on the basis of data of polymerase chain reaction with forward (5'-GGCCTCTTTCATCACAGACC-3') and reverse (5'-AGATGCAGCAAAGCCAAAGT-3') primers. Amplification products were incubated with BsmI restrictase in buffer. Hydrolysis products were isolated in polyacrylamide gel and visualized under ultraviolet light.

Three ADIPOQ genotypes (G/G, G/T and T/T) were identified using G276T polymorphism.

The results were processed by methods of variation statistics using the STATISTICA software. The data are presented as $M \pm \sigma$, where M is the average vaue; σ is the standard deviation. The results of the genetic analysis were evaluated using the $\chi 2$-test and the Fisher method. During the significance analysis of the differences between the two groups in the severity of the index, expressed by a number, the Student's t-test was used.

Results and discussion.

As a result of the study, it was found that the t allele, which, according to a number of researchers [7, 13–15], is associated with the development of IR, occurred in a significantly larger number of patients with comorbidity EH and obesity compared with the group of patients with hypertension with normal body weight ($p = 0.005$) and the control group ($p = 0.005$) (Table 1). It should be noted that in patients with hypertension in the presence of excessive weight allele T also occurred significantly more often than in patients with EH with normal body weight ($p = 0.030$) and healthy individuals ($p = 0.024$).

Table 1 – Distribution of ADIPOQ alleles and genotypes in the study groups

Indicator	Study group				Difference between the groups					
	EH + obesity, n = 200	EH + normal body weight, n = 50	EH + excessive body weight, n = 50	Control group, n = 30	χ^2, p_{1-2}	χ^2, p_{1-3}	χ^2, p_{1-c}	χ^2, p_{2-3}	χ^2, p_{2-c}	χ^2, p_{3-c}
	1	2	3	C						
G/G	76 (38 %)	30 (60 %)	26 (52 %)	20 (66.7 %)	7.93 p = 0.005	3.25 p = 0.072	8.82 p = 0.003	0.65 p = 0.420	0.36 p = 0.551	1.65 p = 0.001
G/T	101 (50.5 %)	19 (38 %)	21 (42 %)	9 (30.0 %)	2.5 p = 0.114	1.16 p = 0.282	4.39 p = 0.036	0.17 p = 0.683	0.23 p = 0.638	1.15 p = 0.283
T/T	23 (11.5 %)	1 (2 %)	3 (6 %)	1 (3.3 %)	p = 0.028	p = 0.192	p = 0.146	p = 0.309	p = 0.712	p = 0.517
G-allele	253 (63.2 %)	78 (78 %)	65 (65 %)	49 (81.7 %)	7.78 p = 0.005	0.11 p = 0.834	7.85 p = 0.005	p = 0.030	031 p = 0.579	5.09 p = 0.024
T-allele	147 (36.8 %)	22 (22 %)	35 (35 %)	11 (18.3 %)						

Analysis of the distribution of genotypes in the study groups showed that homozygous G/G-genotype, which, according to scientists, is considered as protective, was observed in significantly fewer hypertensive obese patients compared with patients with normal body weight (p = 0.005) and healthy individuals (p = 0.003). Also, in hypertensive patients with excessive weight, this genotype was presented in significantly fewer patients compared to the control group (p = 0.001). At the same time, unfavorable (according to some authors) homozygous T/T-genotype occurred in significantly more hypertensive patients with obesity compared to patients with normal body weight (p = 0.028), and heterozygous G/T-genotype – in significantly more obese patients compared to healthy individuals (p = 0.036) (Table 1).

At the next stage of the study, the effect of ADIPOQ polymorphism on the severity of metabolic indicators in patients with comorbidity EH and obesity was assessed (Table 2).

It was found that in patients with EH and obesity in the presence of G/T and T/T-genotypes BMI was significantly higher (p = 0.009 and p = 0.021, respectively) than in G/G-genotype. G/T and T/T-genotypes also showed significantly higher levels of insulin (p = 0.000) and HOMA-IR (p = 0.009 and p = 0.000) than G/G-genotype, which indicates the association of ADIPOQ polymorphism with IR severity and confirms the results of a number of researchers [5, 7–9]. This polymorphism was also associated with the severity of the adipokine imbalance: G/T and T/T-genotypes showed significantly lower levels of adiponectin (p = 0.001 and p = 0.048, respectively) and significantly higher levels of leptin (p = 0.000 and p = 0.003, respectively) compared to the G/G-genotype. The homozygous T/T-genotype was additionally associated with higher triglyceride levels (p = 0.047) compared to the G/G-genotype (Table 2).

Table 2 – Metabolic indicators in patients with comorbidity EH and obesity depending on ADIPOQ genotypes

Indicator	G/G-genotype, n = 76	G/T-genotype, n = 101	T/T-genotype, n = 23
BMI, kg/m^2	33.98 ± 2.94	35.18 ± 2.70*	35.90 ± 1.78**
Fasting blood glucose, mmol/l	4.89 ± 0.26	4.87 ± 0.26	4.92 ± 0.28
Insulin, μAU/ml	11.73 ± 4.26	16.72 ± 4.61*	18.57 ± 5.36**
HOMA-IR	2.54 ± 0.89	3.62 ± 1.01*	4.05 ± 1.18**
Total cholesterol, mmol/l	6.08 ± 0.52	6.11 ± 0.48	5.90 ± 0.34
Triglycerides, mmol/l	1.83 ± 0.34	1.94 ± 0.37	2.05 ± 0.40*
LDL cholesterol, mmol/l	4.71 ± 0.72	4.88 ± 0.49	4.95 ± 0.58
HDL cholesterol, mmol/l	0.99 ± 0.10	1.00 ± 0.10	0.97 ± 0.07
Adiponectin, ng/ml	6.53 ± 0.50	6.41 ± 0.33*	6.33 ± 0.56**
Leptin, ng/ml	13.96 ± 2.47	15.74 ± 2.28*	16.75 ± 2.48**

*Statistically significant differences between G/G and G/T-genotypes;
**Statistically significant differences between G/G and T/T-genotypes.

Taking into account the differences between G/T and T/T-genotypes and G/G-genotype in a number of indicators, as well as the absence of significant differences between G/T and T/T-genotypes, at the further stage of the study, patients with G/T and T/T-genotypes were grouped into G/T- + T/T genotype (Table 3).

Table 3 –Comparative evaluation of metabolic indicators of patients with comorbidity EH and obesity in ADIPOQ genotypes G/G and G/T + T/T

Indicator	First group, n = 200	
	Genotype	
	G/G, n = 76	G/T + T/T, n = 124
BMI, kg/m^2	33.98 ± 2.94	35.32 ± 2.56*
Fasting blood glucose, mmol/l	4.89 ± 0.26	4.88 ± 0.26
Insulin, μAU/ml	11.73 ± 4.26	17.06 ± 4.79*
HOMA-IR	2.54 ± 0.89	3.70 ± 1.05*
Total cholesterol, mmol/l	6.08 ± 0.52	6.09 ± 0.45
Triglycerides, mmol/l	1.83 ± 0.34	2.02 ± 0.39*
LDL cholesterol, mmol/l	4.71 ± 0.72	4.86 ± 0.51
HDL cholesterol, mmol/l	0.99 ± 0.10	1.00 ± 0.10
Adiponectin, ng/ml	6.53 ± 0.50	6.38 ± 0.51*
Leptin, ng/ml	13.96 ± 2.47	15.75 ± 2.31*

*Statistically significant differences between G/G and G/T + T/T-genotypes

Comparative evaluation of G/G-genotype with combined G/T- + T/T-genotype showed similar associations of ADIPOQ genetic polymorphism with the severity of metabolic disorders, as in the separate consideration of three variants of genotypes. Thus, hypertensive patients with obesity in the presence of combined G/T- + T/T-genotype had significantly higher BMI (p = 0.004), higher levels of insulin (p = 0.000), HOMA-IR (p = 0.000), triglycerides (p = 0.049) and leptin (p = 0.000), as well as significantly lower levels of adiponectin (p = 0.005) compared to the G/G-genotype (Table 3).

Thus, the study showed that in patients with EH and concomitant obesity, in contrast to patients with EH and normal body weight and healthy individuals, T allele, which is associated with IR, was observed significantly more often. ADIPOQ genetic polymorphism significantly influenced the metabolic indicators of patients with comorbidity EH and obesity. At the same time, patients with G/T - and T/T-genotypes showed more pronounced metabolic disorders than patients with G/G-genotype.

Conclusions

Association of T allele of polymorphic marker G276T of ADIPOQ gene with development of comorbidity EH and obesity was established.

Genetic polymorphism ADIPOQ influenced changes in metabolic indicators in patients with EH and obesity: G/T and T/T-genotypes showed more pronounced abnormalities compared to G/G-genotype.

G/T and T/T-genotypes of the polymorphic marker G276T of ADIPOQ gene were associated with significantly higher BMI, higher levels of atherogenic lipoproteins (triglycerides), more severe IR and imbalance of adipokines.

Further research perspectives

Considering the important role of ADIPOQ gene polymorphism in the development of IR, it is necessary to note the perspectives of studying the simultaneous effect of polymorphisms of other genes that affect the IR formation.

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