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GENETIC AND BIOCHEMICAL MARKERS OF ENDOTHELIAL DYSFUNCTION IN YOUNG PATIENTS WITH ARTERIAL HYPERTENSION AND CONCOMITANT OBESITY

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The total of 123 young hypertensive patients were examined with normal weight, overweight and obesity (average age 32.83±0.58 years old). They were performed genetic analysis of endothelial NO-synthase gene polymorphisms – T(-786)C and G894T as genetic markers of endothelial dysfunction. The frequency dominance of G894T polymorphism "pathological" genotypes was revealed in all groups. Cases of combination of T(-786)C and G894T polymorphisms were significantly more frequent in young hypertensive patients with concomitant obesity. Homocysteinemia and daily microalbuminuria were checked as biochemical markers of endothelial dysfunction. These markers were more expressed in concomitant overweight and obesity compared with normal-weight young hypertensive patients. A significant positive correlation was revealed between the levels of homocysteinemia and microalbuminuria, and also between these markers and the body mass index. It was found that "pathological" genotypes of both studied eNOS gene polymorphisms are associated with a higher level of homocysteinemia and daily microalbuminuria. arterial hypertension, obesity, homocysteine, microalbuminuria

Key words: endothelial NO synthase gene, polymorphism, homocysteinemia, microalbuminuria, arterial hypertension, obesity.

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ГЕНЕТИЧНІ ТА БІОХІМІЧНІ МАРКЕРИ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ У МОЛОДИХ ПАЦІЄНТІВ З АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ ТА СУПУТНІМ ОЖИРІННЯМ

Обстежено 123 пацієнта молодого віку з артеріальною гіпертензією із нормальною вагою, надмірною масою тіла та ожирінням (середній вік 32,83±0,58 років). Був проведений генетичний аналіз поліморфізмів гена ендотеліальної NO-синтази – T(-786)C та G894T, як генетичних маркерів ендотеліальної дисфункції. Було виявлено частотне переважання «патологічних» генотипів поліморфізму G894T у всіх групах. Випадки комбінації поліморфізмів T(-786)C та G894T були значно частішими у молодих гіпертензивних пацієнтів із супутнім ожирінням. Як біохімічні маркери ендотеліальної дисфункції були визначені гомоцистеїнемія та добова мікроальбумінурія. Ці маркери були більш вираженими при супутній надмірній масі тіла та ожирінні порівняно з молодими гіпертензивними пацієнтами нормальної маси тіла. Було визначену значну позитивну кореляцію між рівнями гомоцистеїнемії та мікроальбумінурії, а також між цими маркерами та індексом маси тіла. Було виявлено, що «патологічні» генотипи обох досліджених поліморфізмів гена eNOS пов'язані з вищим рівнем гомоцистеїнемії та добової мікроальбумінурії.артеріальна гіпертензія, ожиріння, гомоцистеїн, мікроальбумінурія.

Ключові слова: ген ендотеліальної NO-синтази, поліморфізм, гомоцистеїнемія, мікроальбумінурія, артеріальна гіпертензія, ожиріння.

The study is a fragment of the research project "Clinical and pathogenetic features of osteoporosis, its diagnostics, prevention and treatment in comorbid pathology", state registration No. 0121U113936).

Nowadays, cardiovascular diseases (CVD) remain the leading health problem in the world. Detection and correction of risk factors for CVD is a priority of preventive measures, especially in patients with comorbid pathology.

Arterial hypertension (AH), diabetes mellitus, hypercholesterolemia and chronic smoking are the main factors which lead to CVD, that is closely related to the development of endothelial dysfunction (ED), is one of the central pathogenetic mechanisms of vascular damage of various genesis [9].

AH is considered to be a "silent killer", because the compensatory mechanisms are often masked, and the course of the disease is characterized by the absence of clinical manifestations for a long period of time [9, 12]. The SPRINT study has found a significant reduction in cardiovascular events and mortality in high-risk patients which was achieved by decreasing of blood pressure below 120 mm Hg [12]. Many studies have demonstrated the effectiveness of ED identifying markers as an early manifestation and independent predictor of unfavorable prognosis of vascular diseases [9]. Risk factors, particularly AH, the formation of excess free fatty acids or insulin resistance (IR) potentiate the aggressive effect on the vascular wall condition and enhance systemic ED, which in turn leads to aggravation of the vasoconstrictor effect and low bioavailability of nitric oxide (NO) [5, 14].

NO synthesis is catalyzed by nitric oxide synthases (NOS), in turn the endothelial nitric oxide synthase (eNOS) is an isoform of NOS, which is responsible for catalyzing of the NO formation from l-arginine in endothelial cells, playing a significant role in vascular function [5, 14]. Candidate gene

polymorphisms of the micro- and macrovascular pathology of eNOS - G894T, T-786C and 4a/4b – are responsible for changes in NO production and are associated with CVD, including AH, coronary heart disease and type 2 diabetes mellitus [5].

Determination of specific biochemical markers in peripheral blood is an effective method for characterizing of vascular endothelium condition and diagnosis of ED [5].

Thus, microalbuminuria (MAU) is considered to be a certain prognostic marker of future cardiovascular events. In the study by Feldt-Rasmussen et al. the relationship between increased urinary albumin excretion, markers of low-grade inflammation and generalized ED has been demonstrated [7].

Another indicator of ED is the determination of homocysteine (HC) levels, as a number of studies have found an association between hyperhomocysteinemia (hyperHC) and the development of CVD [2]. In patients with MAU and hyperHC, modulation of endothelial function may lead to a reduction in cardiovascular events and be a therapeutic target for primary prevention [7].

Despite of significant achievements in the diagnosis and treatment of cardiovascular and cerebrovascular diseases, it remains actual the search for targeted, preventive measures for early, personalized modification of predictors in patients of risk groups by identifying of biochemical markers of the CVD initial stages.

The purpose of the work was to study the prevalence and genotypes distribution of T(-786)C and G894T polymorphisms of the endothelial NO-synthase gene, to study the features of endothelial dysfunction by determining of the homocysteinemia level and daily microalbuminuria in young patients with arterial hypertension, depending on the presence or absence of concomitant obesity (or overweight). Patients were examined in the cardiology department of the Multidisciplinary Medical Center of the Odessa National Medical University, Ukraine.

Materials and methods. 123 young patients with essential arterial hypertension (18–44 years old) were examined, the average age was (32.83±0.58) years old, and the male/female ratio was 72/51 respectively. For all patients the body mass index (BMI) was calculated using the Quetelet's formula: body weight/height^2 (kg/m^2). Depending on BMI, all patients were divided into 3 groups: group 1 (n=41) consisted of patients with normal body weight, BMI=18.5–24.9 kg/m^2; group 2 (n=40) consisted of patients with overweight, BMI=25–29.9 kg/m^2; group 3 (n=42) – patients with obesity, BMI≥30 kg/m^2.

The research was performed with bilateral signing of an informed agreement form, in accordance with the principles of the Helsinki Declaration of Human Rights, Council of Europe Convention on Human Rights and Biomedicine (ETS-164) from 04.04.1997.

All patients were performed polymerase chain reaction (PCR) analysis of two polymorphisms of the endothelial NO-synthase gene – T(-786)C and G894T. Venous whole blood samples were used as the test material. Genomic DNA was isolated using the Blood Genomic DNA Extraction kit-50; 100. PCR analysis of the CYP11B2 gene was performed on an amplifier with a fluorescent signal detection system in the real-time CFX96 (Bio-Rad, USA). According to the results of PCR, one of three possible genotypes was identified for each polymorphism of the eNOS gene: polymorphism T(-786)C – genotypes TT, TC, CC; polymorphism G894T – genotypes GG, GT, TT. For obtaining of more significant statistical results during comparing the frequency of genotypes occurrence, "pathological" genotypes for each polymorphism of the eNOS gene were combined (dominant model) – genotypes (TC+CC) of the T(-786)C polymorphism and genotypes (GT+TT) of the G894T polymorphism. Also, it was formed the separate group of examined patients with combination of "pathological" genotypes of both studied polymorphisms of the eNOS gene. The frequencies of genotypes occurrence of the eNOS gene polymorphisms T(-786)C and G894T in three examined groups were analyzed.

ED in the examined patients was studied on the basis of biochemical parameters such as blood homocysteine levels and daily microalbuminuria. The blood HC level was determined by the immunochemiluminescence method on the automatic chemiluminescence analyzer Immulite 2000 (Siemens Healthcare Diagnostics Inc., USA). The concentration of albumin in daily urine (MAU) was tested by the immunoturbidimetric method on the automatic biochemical analyzer AU-480 (Beckman Coulter Inc., Japan). The reference values for MAU were considered the level of albumin in daily urine 30.0–299.0 mg/day.

Processing of the obtained results was carried out by statistical software programs Statgraphics Plus 5.0, Statistica 10.0. The frequencies of genotypes of the eNOS gene polymorphisms T(-786)C and G894T were studied in the absolute and relative (%) values; the correspondence of the alleles and genotypes distribution to the Hardy-Weinberg principle was analyzed. Quantitative parameters were presented as average value (M), average error (m) and 95 % confidence interval (95 % CI). A comparative analysis of the genotype frequencies of the studied gene between the groups was

performed using two-sided Fisher's exact test. The parametric Student's t-test and nonparametric Mann-Whitney U-test were used to assess the significance of differences in quantitative parameters between groups. Correlation-regression analysis was carried out with the determination of the Spearman correlation coefficient (r), the linear regression equation and linear determination coefficient (r^2). The results were considered statistically significant when $p<0.05$.

Results of the study and their discussion. In the research, the frequency of genotypes of eNOS gene polymorphisms T(-786)C and G894T was analyzed, as well as the features of endothelial dysfunction according to homocysteine level and microalbuminuria, their association with studied genetic polymorphisms. Analyzing the obtained data, the following results were revealed. Table 1 shows the frequency of genotypes of eNOS gene polymorphisms T(-786)C and G894T in young hypertensive patients with normal weight, overweight and obesity.

Table 1

Distribution of genotypes of the eNOS gene polymorphisms T(-786)C and G894T in young patients with arterial hypertension, depending on the presence or absence of concomitant obesity (or overweight), abs./%

Genotype of the eNOS gene	Group 1 AH with normal BMI (n=41)	Group 2 AH with overweight (n=40)	Group 3 AH with obesity (n=42)	All patients (n=123)	p
Polymorphism T(-786)C					
"Normal" genotype TT, abs./%	15 (36.6 %)	22 (55.0 %)	20 (47.6 %)	57 (46.3 %)	p1=0.12 p2=0.38 p3=0.52
"Pathological" genotypes TC+CC, abs./%	26 (63.4 %)	18 (45.0 %)	22 (52.4 %)	66 (53.7 %)	
Polymorphism G894T					
"Normal" genotype GG, abs./%	18 (43.9 %)	12 (30.0 %)	12 (28.6 %)	42 (34.1 %)	p1=0.25 p2=0.17 p3=0.62
"Pathological" genotypes GT+TT, abs./%	23 (56.1 %)	28 (70.0 %)	30 (71.4 %)	81 (65.9 %)	
Polymorphisms T(-786)C+G894T					
Combination of "pathological" genotypes, abs./%	10 (24.4 %)	14 (35 %)	20 (47.6 %)	44 (100 %)	p1=0.33 p2=0.04 p3=0.27

Note: p1 – significance of differences between 1st and 2nd groups; p2 – significance of differences between 1st and 3rd groups; p3 – significance of differences between 2nd and 3rd groups.

The distribution of the genotypes of the T(-786)C polymorphism was as follows: in patients with AH and normal BMI (group 1) the "normal" TT genotype was detected in 15 patients (36.6 %), in patients with AH and overweight (group 2) the TT genotype was found in 22 (55.0 %), in patients with AH and concomitant obesity – in 20 (47.6 %). The frequency of "pathological" genotypes (TC+ CC) of T(-786)C polymorphism in group 1 was 63.4 % (26 patients), in group 2 – 45.0 % (18 patients), in group 3 – 52.4 % (22 patients). Totally the frequencies of occurrence of the TT genotype and genotypes (TC+CC) were 57 (46.3 %) and 66 (53.7 %) patients respectively. That is, in all examined groups of young hypertensive patients there were no significant differences (p=0.073) between the prevalence of "normal" and "pathological" genotypes of T(-786)C polymorphism of the eNOS gene. Comparing the frequencies of genotypes occurrence between groups, there were no significant differences, i.e. the distribution of genotypes frequencies of the studied polymorphism does not significantly influence on the BMIs.

The results, obtained in regard to the second studied polymorphism G894T of the eNOS gene, revealed other tendencies differing from the polymorphism T(-786)C. The frequency of "pathological" genotypes (GT+TT) of the G894T polymorphism in the examined groups exceeded the frequency of the "normal" GG genotype, that was not observed in the T(-786)C polymorphism of the eNOS gene. So, in group 1 the GG genotype was found in 18 (43.9 %) patients, genotypes (GT+TT) – in 23 (56.1 %) patients. In groups 2 and 3 these differences were even more expressed (p=0.001). In group 2, the GG genotype was found in 12 (30.0 %) patients, genotypes (GT+TT) – in 28 (70.0 %) patients; in group 3 – 12 (28.6 %) versus 30 (71.4 %) patients respectively. Totally, in all patients the frequency of GG genotype was 34.1 % of patients, genotypes (GT+TT) – 65.9 % of patients (p=0.022). Comparing the frequency of genotypes between groups, no significant differences were obtained, i.e. the prevalence of G894T polymorphism genotypes did not differ significantly in young hypertensive patients with different BMI values.

In the particular part of the examined patients (44/35.8 %) it was revealed a combination of "pathological" genotypes of both studied polymorphisms of the eNOS gene. Wherein, the frequency of

such combination was significantly different only between groups 1 and 3: 24.4 % versus 47.6 % (p2=0.04).

Analyzing such a biochemical marker of ED as homocysteine, hyperhomocysteinemia (>15 µmol/l) was detected in 44 (35.7 %) patients: group 1 – 10 (8.1 %), group 2 – 15 (12.2 %), group 3 – 19 (15.4 %) patients. According to the results of the study (table 2), the average value of the level of HC in group 1 was 11.48 [95 % CI: 10.3; 12.7] µmol/l, in group 2 – 13.5 [95 % CI: 12.7; 14.3] µmol/l, in group 3 – 15.64 [95 % CI: 14.8; 16.5] µmol/l.

Table 2

Homocysteinemia and daily microalbuminuria as endothelial dysfunction markers in young patients with arterial hypertension, depending on the presence or absence of concomitant obesity (or overweight), M [95 % CI]

Parameters	All young patients with AH (n=123)			p
	Group 1 AH with normal BMI (n=41)	Group 2 AH with overweight (n=40)	Group 3 AH with obesity (n=42)	
Homocysteine, µmol/l	11.48 [10.3; 12.7]	13.5 [12.7; 14.3]	15.64 [14.8; 16.5]	p1=0.007 p2=0.000 p3=0.000
Daily microalbuminuria, mg/day	18.61 [15.5; 21.7]	23.11 [19.5; 26.7]	28.66 [22.9; 34.4]	p1=0.024 p2=0.004 p3=0.321

Note: p1 – significance of differences between 1st and 2nd groups; p2 –significance of differences between 1st and 3rd groups; p3 – significance of differences between 2nd and 3rd groups.

Comparing the average concentration of HC in the blood between all groups, there were revealed significant differences (p1=0.007, p2=0.000, p3=0.000).

In addition to the level of HC, daily albuminuria was studied in the examined young hypertensive patients as a biochemical marker of ED. MAU (30.0299.0 mg/day) was revealed totally in 30 (24.4 %) patients: group 1 6 (4.9 %), group 2 9 (7.3 %), group 3 15 (12.2 %) patients. Evaluating the average value of this parameter in groups, it was revealed that it increases with the growth of BMI in the groups. In group 1 the average daily MAU was 18.61 [95 % CI: 15.5; 21.7] mg/day, in group 2 – 23.11 [95 % CI: 19.5; 26.7] mg/day, in group 3 – 28.66 [95 % CI: 22.9; 34.4] mg/day. At the same time, significant differences were between groups 1 and 2 (p1=0.024), groups 1 and 3 (p2=0.004).

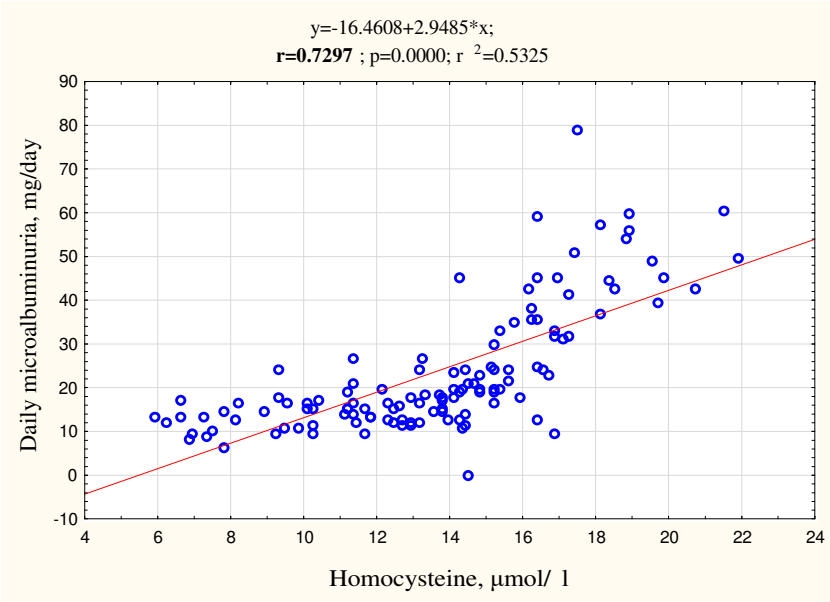

Fig.1. Correlation between the level of homocysteinemia and daily microalbuminuria in young patients with arterial hypertension

In our study a correlation-regression analysis was performed between the levels of HC, daily MAU and BMI in young patients with AH. Between the HC level and BMI there was revealed a direct positive correlation of moderate strength (r=0.51, p=0.000). Between MAU and BMI – a direct positive correlation of weak strength (r=0.39, p=0.000). Also it was studied the correlation relationship between the level of HC and MAU, as two biochemical markers of ED (fig. 1). A strong direct positive correlation was found (r=0.73, p=0.000).

In the research it was studied the association of "normal" and "pathological" genotypes of T(-786)C and G894T polymorphisms of the eNOS gene with the level of HC and daily MAU in young hypertensive patients (fig. 2).

Thus, the mean blood HC value was significantly higher in patients with isolated "pathological" genotypes of the T(-786)C and G894T polymorphisms of the eNOS gene compared with the normal genotypes of these polymorphisms. In patients with isolated T(-786)C polymorphism of the eNOS gene (TC+CC genotypes), the mean HC was (15.30±0.41) µmol/l versus patients with

"normal" TT genotype, where the mean value of this parameter was (11.54±0.34) μmol/l (P=0.000). In patients with isolated G894T polymorphism (GT+TT genotypes), the mean HC was also significantly higher compared with the "normal" GG genotype: (14.22±0.41) versus (12.28±0.42) μmol/l respectively (P=0.002). However, the highest mean value of HC concentration in the blood was revealed in combination of "pathological" genotypes of both studied polymorphisms of the eNOS gene (in 44/35.8 % patients): the mean HC was (16.69±0.36) μmol/l. During comparative analysis of this parameter between groups of patients with isolated and combined "pathological" genotypes of both polymorphisms, the most significant differences were found between the combination of T(-786)C and G894T polymorphisms of the eNOS gene and each isolated polymorphism separately (p=0.012 and p=0.000 respectively).

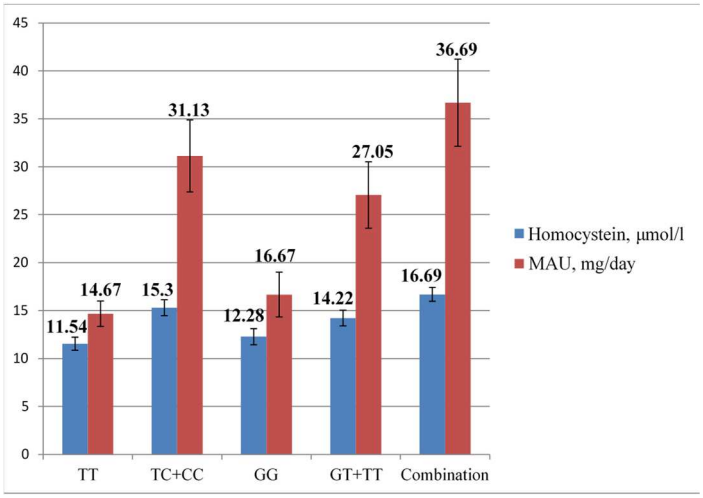

Fig. 2. Association of genotypes of the eNOS gene T(-786)C and G894T polymorphisms with the level of homocysteinemia and daily microalbuminuria, as biochemical markers of endothelial dysfunction, in young patients with arterial hypertension

Analyzing the mean values of daily MAU, in carriers of genotypes (TC+CC) of isolated polymorphism T(-786)C of the eNOS gene, this parameter was significantly higher compared with carriers of genotype TT - (31.13±1.88) versus (14.67±0.66) mg/day respectively (P=0.000). A similar trend with high significance was revealed in regard to the G894T polymorphism (P=0.001). However, as in the case of homocysteinemia, the highest mean value of daily MAU was observed in combination of both T(-786)C and G894T polymorphisms of the eNOS gene and was (36.69±2.27) mg/day, with a significant difference in comparison with each isolated polymorphism separately (p=0.000).

The obtained distribution of the of the T(-786)C polymorphism genotypes of the eNOS gene has showed that the "normal" TT genotype and the "pathological" genotypes (TC+CC) were equally often in the examined young hypertensive patients. Moreover, this distribution of genotypes in the patient groups did not differ depending on the BMI. According to the literature, the exact role of T(-786)C genetic polymorphism and its genotypes in the development of AH remains ambiguous. Some studies indicate that the C allele of this polymorphism increases the risk of AH and its pathophysiological components. [3]. Other studies indicate a greater role of this polymorphism in the development of coronary heart disease [8]. The higher prevalence of genotypes (GT+TT) of the eNOS gene G894T polymorphism in young hypertensive patients, revealed in our study, coincided with the data of most other studies of this polymorphism and its role in the pathogenesis of AH [1].

Analyzing the level of homocysteine as biochemical marker of ED, the presence of concomitant obesity significantly affects on hyperHC, which has been demonstrated in other studies [6]. The exact mechanism of increasing of the HC concentration in obesity according to the available scientific literature is not fully studied. HyperHC in obesity can be caused, on the one hand, by insulin resistance and the negative effect of insulin on hepatic transsulfation of HC [15], on the other hand, by the effect of HC on insulin resistance due to a resistin-dependent mechanism, which is activated by adipose tissue [10]. The obtained results of daily albuminuria as another ED marker, indicating on the effect of obesity and overweight on MAU, coincide with the results of other researches, which studied the independent role of obesity in increasing of urinary albumin excretion [13].

The obtained data indicate a high association of eNOS genetic polymorphisms with homocysteine levels as a biochemical marker of ED, which coincides with the results of other studies [4] and allows to consider the T(-786)C and G894T polymorphisms of the eNOS gene as a genetic marker of ED and hyperHC. The revealed association of the studied eNOS gene polymorphisms with MAU coincides with the researches of other authors [11].

Despite the presence of data on the effect of isolated T(-786)C and G894T polymorphisms of the eNOS gene on various markers of ED, in available scientific sources there are do no data on the effect of the combined variant of the occurrence of T(-786)C and G894T polymorphisms of the eNOS gene on endothelial dysfunction in hypertensive patients.

Conclusions

1. In the examined young patients with arterial hypertension, "normal" and "pathological" genotypes of the eNOS gene T(-786)C polymorphism were equally often, while there were no significant differences between groups with different body mass indexes. The distribution of the G894T polymorphism genotypes of the eNOS gene was characterized by the high prevalence of "pathological" genotypes (GT+TT), while there were also no significant differences between the groups with normal body weight, overweight and obesity. Cases of combination of T (-786) C and G894T polymorphisms were significantly more frequent in young hypertensive patients with concomitant obesity.

2. Based on such biochemical markers of ED as homocysteinemia and daily microalbuminuria, a more expressed endothelial dysfunction was revealed in concomitant overweight and, mostly, obesity in young hypertensive patients. It was revealed a significant positive correlation between the levels of homocysteinemia and microalbuminuria, and also between these ED markers and BMI. Therefore, the determination of blood homocysteine concentration and daily microalbuminuria can be recommended for assessment of the functional condition of the endothelium in hypertensive young patients, especially with concomitant obesity and overweight.

3. "Pathological" genotypes of both polymorphisms T(-786)C and G894T of the eNOS gene are associated with a higher level of homocysteinemia and daily microalbuminuria as biochemical markers of endothelial dysfunction. In simultaneous combination of "pathological" genotypes of these eNOS gene polymorphisms in young hypertensive patients endothelial dysfunction is more severe and can be considered as genetic ED marker.

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