STRUCTURAL AND FUNCTIONAL CONDITION OF THE HEART IN PATIENTS WITH ARTERIAL HYPERTENSION DEPENDING ON A1166C-GENE POLYMORPHISM OF ANGIOTENSIN II TYPE 1 AND T786C-PROMOTER OF ENDOTHELIAL NO-SYNTASE GENE

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Key words: arterial hypertension, gene polymorphism
Ключові слова: артеріальна гіпертензія, генний поліморфізм

Abstract. Structural and functional condition of the heart in patients with arterial hypertension depending on A1166C-gene polymorphism of angiotensin II type 1 and T786C-promoter of endothelial NO-synthase gene.

Hnizdiukh R.V., Shmanko V.V. It's known that pathological changes in structural and functional condition of the heart, which are caused by arterial hypertension, trigger development of chronic heart failure and disablement of population. Nowadays, it proven that candidate genes, expression products of which participate in regulating vascular tone, have considerable influence upon development of arterial hypertension, however their role in pathogenesis of arterial hypertension has not been fully clarified, and results of the respective researches significantly vary among different populations. To determine the structural and functional state of the heart in patients with hypertension depending on the polymorphism of the A1166C gene of the angiotensin II receptor type I and the T786C promoter of the endothelial NO-synthase gene we examined 86 patients, aged from 45 to 76 years. 30 people without signs of hypertension were in the control group. The structural and functional state of the heart was assessed by cardiac ultrasound according to standard methods. Studies of the A1166C allelic polymorphism of the angiotensin II receptor gene type I and the T786C promoter of the eNOS gene were performed by polymerase chain reaction with electrophoretic detection of results. Analysis of cardiac ultrasound showed, that in the patients – carriers of C-allele of both studied genes (AC+CC and TT+CC) left ventricular ejection fraction tended to decrease. We found a bigger thickness of the posterior wall of the left ventricle in patients with CC genotype compared to carriers of AA genotype A1166C – (1.3±0.07) cm vs. (1.1±0.05) cm (p<0.005). The mass of the left ventricular myocardium index in the group of patients with the genotype AC and CC was (157.5±7.3) g/m² and (161.5±7.1) g/m², respectively, being by 16.7% and 19.6% more than in carriers of AA genotype.

The research shows that inheritance of CC genotype for A1166C polymorphism of the angiotensin II type 1 receptor gene and of CC T786C polymorphism in the promoter of eNOS gene is associated with more noticeable changes in structural and functional heart condition among patients with arterial hypertension.

Refeřat. Структурно-функциональний стан серця у хворих на артеріальну гіпертензію залежно від поліморфізму A1166C-гена рецептора ангиотензину II першого типу та T786C-промотора гена ендотеліальної NO-синтази. Гніздюх Р.В., Шманько В.В. Відомо, що патологічні зміни в структурно-функціональному стані серця, які виникають ушкоджування артеріальної гіпертензії, є пусковим механізмом для розвитку хронічної серцевої недостатності та інвалідизації населення. На сьогодні доведено, що при експресії ряду генів-кандидатів утворюються речовини, які беруть участь у регуляції тонусу судин, проте їх роль у
Cardiovascular diseases (CVD) are the most common in Ukraine, so the pathology of the circulatory system affects about 22.3 million people, ie 52.4% of the population [1]. Arterial hypertension (AH) occupied the leading place in the structure of cardiovascular diseases [2]. Experts from the World Health Organization (WHO) note that hypertension is the most significant cause of death and disability among all CVDs [3].

The life and health prognosis of patients with hypertension largely depends on the effect of elevated blood pressure (BP) on target organs [4]. An uncontrolled BP level is the cause of left ventricular hypertrophy (LVH), which in turn leads to an increase in the LV myocardial mass (MM) and, as a result, an increase in the oxygen demand of the heart muscle and an increase in the risk of ectopic murmurs, dysmetabolic changes, as well as emergence and progression of diastolic and systolic dysfunctions [5].

It is known that a combination of hereditary factors and negative environmental influences plays an important role in the development of hypertension [6]. In recent years, influence of various genes on the development of hypertension has attracted considerable attention of medical science [7]. According to the hemodynamic theory of the hypertension development, a key role in its pathogenesis is played by an imbalance between the action of agents involved in the vasoconstriction and vasodilatation of blood vessels. Substances involved in the regulation of vascular tone are products of the expression of certain genes, thus, the human genotype can play an indirect role in the pathogenesis of hypertension. Such genes include the type I angiotensin gene and endothelial NO synthase [8].

The efforts of scientists from all over the world are aimed at studying the role of gene mutations in the pathogenesis of hypertension, however, as evidenced by research results, the prevalence of such mutations differs in different populations [9].

The purpose of this study was to find out the structural and functional state of the heart in patients with hypertension depending on the polymorphism of the A1166C gene of the angiotensin II receptor type I and the T786C promoter of the endothelial NO synthase gene.

MATERIALS AND METHODS OF RESEARCH

86 patients with arterial hypertension aged 45-76 (47 (55%) women and 39 (45%) men) took part in the study. The average age was (61.35±13.3) years; Treatment and examination were carried out in the Therapeutic Department of the Kozova Central Hospital. The control group consisted of 30 patients without signs of hypertension.

Family history regarding the early development of CVD (<55 years for men, <65 years for women) was found in 47 patients (55%). At the time of inclusion in the study, 27 people (31%) were active smokers, and 11 persons abused alcohol, which made up 13%. 30 patients (35%) suffered from obesity. Among the examined patients, 19 (22%) had hypertension stage I, and 67 (78%) had stage II. The study included patients diagnosed with hypertension 1-3 degree: 24% – degree 1, 55% – degree 2, and 21% – degree 3. The average daily blood pressure level by group is shown in Table 1.

The diagnosis of hypertension was established in accordance with the orders of the Ministry of Health of Ukraine No. 54 and 384 and the Recommendations of...
the Ukrainian Association of Cardiologists on the prevention and treatment of hypertension by evaluating the anamnesis data, complaints, and the results of physical and clinical-instrumental examination.

Exclusion criteria were the presence of hypertension stage III (myocardial infarction and/or stroke in the anamnesis), secondary hypertension, any heart defects, rhythm and conduction disturbances, heart failure of functional class III–IV according to the NYHA, as well as severe concomitant diseases (chronic obstructive pulmonary disease, diabetes, chronic kidney disease, oncological and mental pathologies).

### Table 1

Average daily level of blood pressure by patient groups (M±m)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group</th>
<th>AGTR1 (AA)</th>
<th>AGTR1 (AC)</th>
<th>AGTR1 (CC)</th>
<th>eNOS (TT)</th>
<th>eNOS (TC)</th>
<th>eNOS (CC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>122.63±2.36</td>
<td>138±2.73</td>
<td>148.5±2.66</td>
<td>159±1.52</td>
<td>141±1.9</td>
<td>147.3±2.1</td>
<td>162.5±1.9</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>75.9±1.53</td>
<td>85±1.96</td>
<td>89.75±1.69</td>
<td>109.96±1.39</td>
<td>90±1.72</td>
<td>93.28±2.3</td>
<td>107.25±2.8</td>
</tr>
</tbody>
</table>

Notes: SBP – systolic blood pressure; DBP – diastolic blood pressure.

At the time of inclusion in the study, 63 patients were taking regular antihypertensive therapy, which was 73%. 43% of patients who constantly took antihypertensive drugs reached the target blood pressure level.

When performing the study, the principles of bioethics outlined in the Declaration of Helsinki "Ethical principles of medical research involving people", the "General Declaration on Bioethics and Human Rights (UNESCO)" and the order of the Ministry of Health of Ukraine "On approval of the procedure for conducting clinical trials of medicinal products and examination of materials of clinical tests and standard regulations on ethics commissions" No. 690 of September, 23, 2009. Written informed consent for the study was obtained from all patients. (Conclusion of the Commission on Biomedical Ethics of I. Horbachovsky Ternopil National Medical University No. 69 of April 12, 2022).

The structural and functional state of the heart was studied on the Acuson Sequoia 512 device according to the standard method according to the recommendations of the American Society of Echocardiography and the European Association of Echocardiography [10, 11]. Left ventricular wall thickness (LVWT) and interventricular septal thickness (IVST), and left atrial size (LAS) were determined using ultrasound of the heart. Left ventricular myocardial mass (LVMM) was determined using linear measurements, followed by calculation of the LVMM index (LVMMi) according to the Penn Convention formula. The state of LV systolic function was assessed by the ejection fraction (EF) indicator. Diastolic dysfunction of the left ventricle was assessed in all patients by Doppler echocardiography, taking into account the maximum speed of early (E, cm/s) and late (A, cm/s) diastolic filling of the LV, their ratio (E/A c.u.). A sign of diastolic disorders was considered to be a value of the E/A ratio of less than 1 c.u.

The polymerase chain reaction method with electrophoretic detection of the results was used to study the allelic polymorphism A1166C of the angiotensin II receptor gene type I and T786C – the promoter of the eNOS gene using SNP-EXPRESS reagent sets ("Litech" Ltd. LLC, RF). The frequency of distribution of polymorphic genes in the population was checked according to the Hardy-Weinberg law of genetic equilibrium.

Daily blood pressure monitoring was performed on a portable device SDM23 (Ukraine) according to a standard protocol. The indicators were analyzed using the software of this device. Monitoring took place every 15 minutes during the day and every 30 minutes during the night (every 30 minutes from 10:00 p.m. to 6:00 a.m.).

Statistical analysis of the results was carried out using the Statistica 10 program package ("Statsoft", USA, license number CDDR415X254504GVZ7) and the package of statistical functions of Microsoft Office Excel 2016 (Microsoft Corp., USA). The average arithmetic value (M) and its error (m) were determined. Checking the distribution of samples for normality was carried out according to the Shapiro-Wilk test. The reliability of changes in the average values of the study results between groups was calculated according to the Student's t-test with a normal distribution of independent samples. Statistically significant differences were considered to be p<0.05 [12].

**RESULTS AND DISCUSSION**

The structural and functional state of the heart in patients with hypertension depending on the
polymorphism $A1166C$ and $T786C$, as well as healthy individuals are shown in Table 2.

When analyzing the indicators of systolic function of the myocardium, we did not find a significant difference between patients with hypertension and the control group, however, a tendency to a lower level of LVEF was observed in the carriers of the C-allele of both studied genes (AC+CC and TC+CC).

**Table 2**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genotypes, n=86</th>
<th>Indicator value (M±m) in patients depending on gene polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=30)</td>
<td>EF,%</td>
</tr>
<tr>
<td></td>
<td>AA (n=18)</td>
<td>66.2±2.4</td>
</tr>
<tr>
<td></td>
<td>AC (n=38)</td>
<td>62.3±3.9</td>
</tr>
<tr>
<td></td>
<td>CC (n=30)</td>
<td>58.1±3.4</td>
</tr>
<tr>
<td></td>
<td>eNOS TT (n=24)</td>
<td>64.4±3.5</td>
</tr>
<tr>
<td></td>
<td>TC (n=31)</td>
<td>62.7±3.1</td>
</tr>
<tr>
<td></td>
<td>CC (n=31)</td>
<td>58.4±4.6</td>
</tr>
</tbody>
</table>

Notes: p – the difference between the indicators is significant compared to the control group; p_1 – the difference is significant in compared to with homozygotes by a single gene (AA, TT).

In patients with hypertension LVWT and IVST was significantly higher than in almost healthy individuals (p<0.01). We also found a bigger LVWT in patients with CC genotype compared to carriers of AA genotype $A1166C$ – (1.3±0.07) cm vs. (1.1±0.05) cm (p<0.02).

LV myocardial infarction is considered an independent risk factor for overall and cardiac mortality, as well as the development of cardiovascular events such as myocardial infarction or stroke [13]. The study found that in patients with hypertension, the inheritance of AC and CC $A1166C$ genotypes of the angiotensin II receptor gene type 1 and TC and CC $T786C$ – eNOS promoter is characterized by more significant changes in LV MMi than in carriers of AA and TT genotypes. Thus, LV MMi in the group of patients with the genotype AC and CC was (157.5±7.3) g/m² and (161.5±7.1) g/m², respectively, that is by 16.7% and 19.6% more than in carriers of AA genotype of the $AGTR1$ gene. It should be noted that the difference in LV MMi in carriers of AA and CC genotypes was significant (p<0.03). Our results are consistent with the results of A.S. Shalimov, who established a probable increase in LVMMi in hypertensive patients in the presence of the C-allele of the $AGTR1$ gene [14].

Regarding the $T786C$ polymorphism, in the groups of patients – carriers of C-allele (TC+CC) LV MMi values were (155.2±11.4) g/m² and (158.4±7.9) g/m², respectively, which is 5.4% and 7.5% more than in carriers of TT genotype. The results of numerous experimental studies show that in carriers of the C allele there is a decrease in the expression of the eNOS synthase gene and, as a result, a decrease in the production of nitric oxide, which is known to be involved in the regulation of vascular tone [15].

The mean sizes of LA were significantly higher in patients who had AC and CC genotype of the $AGTR1$ gene, as well as TC and CC genotype of the $eNOS$ gene compared with the control group (Table 2). Comparing the sizes of LA we found a significant difference (p<0.001) in patients-carriers of C-allele (AC+CC genotype) of the $A1166C$ polymorphism of
the AGTR1 gene and in patients – homozygous by the A allele.

We also analyzed the parameters of transmittral blood flow, which are known to be a marker of the left ventricular diastolic function.

We were able to find that patients – carriers of AA AGTR1 gene genotype and homozygotes with the T allele of the eNOS gene did not have diastolic dysfunction. While the severity of diastolic dysfunction was higher in carriers of the CC genotype of the AGTR1 gene and the eNOS gene than in heterozygotes of the studied genes by 4.3% and 3.3%, respectively, which may indicate a relationship between the C allele and the development of diastolic dysfunction in patients with hypertension.

CONCLUSIONS
1. In patients with hypertension, inheritance of AC and CC genotypes of the AGTR1 gene is characterized by significant changes in the structural and functional state of the heart than inheritance of the AA genotype. Homozygotes by C allele (CC genotype) shown significantly higher rates of the left ventricular wall thickness, left ventricular myocardial mass index and the left atrial size compared with patients with AGTR1 AA genotype (p<0.05).

2. In patients with hypertension who were detected a genotype with the presence of the C allele of the eNOS gene, significantly higher values left ventricular myocardial mass index and left atrial size were observed in comparison with the control group (p<0.01).

3. In patients with hypertension, residents of Ternopil region, inheritance of genotype CC polymorphism A1166C of angiotensin II receptor gene type 1 and CC polymorphism of T786C-promoter of eNOS gene is associated with greater changes in left ventricular diastolic dysfunction compared with heterozygotes.

Contributors:
Hniziukh R.V. – conceptualization, methodology, verification, formal analysis, research, writing – original draft, writing – review and editing, visualization;
Shmanko V.V. – conceptualization, methodology, verification, writing – review and editing, supervision.

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