Introduction

Hypertension (AH) is one of the most common diseases of the cardiovascular system. More than 1.5 billion people worldwide suffer from AH but almost 45% of patients are unaware that they have high blood pressure (BP). In Ukraine there are more than 13 million people with AH of whom only 14% receive regular treatment and 35% periodically take medication. Every year this disease is detected in 1 million Ukrainians [11].

Recent studies have convincingly demonstrated the important and independent role of the endothelium in the development of cardiovascular disease (CVD). Many experimental and clinical researches have been published on the role of endothelial dysfunction (ED) in the occurrence and progression of atherosclerosis, AH, congestive heart failure (CHF), coronary heart disease, pulmonary hypertension, myocardial infarction and others [21]. Manifestations of ED are associated by some researchers with an imbalance between the production of vasodilating and vasoconstrictor, prothrombotic, proliferative factors on the one hand (C-natriuretic peptide (CNP), nitric oxide, prostacyclin, tissue activator plasminogen) and vasoconstrictor, prothrombotic, proliferative factors on the other (endothelin-1 (ET-1), superoxidanion, thromboxane A2, tissue plasminogen activator inhibitor) [8].

The endothelium is involved in the pathological process at the earliest stages of AH and CHF [20]. It is proved that ET-1 is one of the markers of AH severity which is determined by the increase in its content in peripheral blood [5, 19, 22, 24, 25]. No less important than the control of ET-1 concentration in almost healthy individuals, in patients with hypertension and CHF is the concentration of a direct antagonist of ET-1 CNP. C-type natriuretic peptide belongs to the family of cardiovascular hormones which include atrial natriuretic peptide (ANP) and natriuretic peptide B-type (BNP). Unlike A- and B- natriuretic peptides which are mainly produced in cardiomycocytes, CNP is an endothelial product synthesized in several vascular channels including the heart and is released in response to stimuli including
vasoconstriction and cytokines, inhibit the proliferation of cardiac fibroblasts, collagen synthesis and myocardial fibrosis [7, 23], inhibit the proliferation and migration of vascular smooth muscle [4, 12], induce coronary vasodilation, regulate vascular homeostasis, promote relaxation of cardiomyocytes [16], stimulate endothelial cells [15]. Studies in patients with CVD have shown that CNP is elevated in AH [19, 22, 24] atherosclerotic lesions [3] and in patients with CHF and has prognostic value in patients with preserved ejection fraction [13]. Thus these benefits of action observed in experimental studies indicate a number of potential mechanisms by which CNP can protect against the progression of CVD, in particular AH and is a marker of the severity of its course.

One of the most studied expression regulators of ET-1 is the polymorphism of the ET-1 gene which is based on the replacement of the amino acid lysine (Lys) by asparagine (Asn) at position 198 of the polypeptide chain which plays an important role in the formation and development of AH and its complications. Carriers of the Asn/Asn genotype of the ET-1 gene showed an increased level of ET-1 expression in plasma compared to carriers of the Lys/Lys genotype [18]. However, the question of the level of CNP in individuals with AH - carriers of polymorphic variants of the ET-1 gene remains open.

The aim of the study to improve the detection of men with AH with persistent long-term increase in blood pressure by using the plasma level of CNP and taking into account the carrier of polymorphic variants of the ET-1 gene.

Materials and methods

The study involved 191 men residents of the Podilia region in Ukraine aged 40-60 years of whom 79 were a control group. The first group included 62 people with AH and LVH (asymptomatic lesion of the target organ), second group - 50 patients with AH and CHF II-III classes according to NYHA.

All persons from the control group and AH were treated in the therapeutic and neurological departments of Vinnytsya Regional Specialized Clinical Dispensary for Radiation Protection of the Ministry of Health of Ukraine and Military Medical Clinical Center of the Central Region of the Air Force of Ukraine (Vinnytsya) and observed on an outpatient basis in the period from December 2013 to June 2014.

Criteria for exclusion from all study groups - the presence of chronic obstructive pulmonary disease, tumors, renal, hepatic, endocrine diseases, diseases of the blood system.

All subjects in the control group as well as patients from the first and second groups of the study were surveyed according to the survey card and after signing informed consent to participate in the research and personal data processing.

The control group included individuals whose results of objective and general clinical examination did not reveal pathological changes in the cardiovascular system including AH.

Criteria for inclusion in the AH study groups were: verified diagnosis of AH with LVH, exclusion of symptomatic hypertension, myocardial infarction, acute cerebrovascular accident and coronary heart disease, the development of which preceded the onset of hypertension.

The diagnosis of AH was established on the basis of patients' complaints, anamnesis and physical examination data, laboratory and instrumental research methods in accordance with the Recommendations of the Ministry of Health of Ukraine dated 24.05.2012 №384. All patients were individually prescribed basic therapy in accordance with the Unified Clinical Protocol for Hypertension approved by the Order of the Ministry of Health of Ukraine dated 24.05.2012 №384 and Clinical guidelines for hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology 2018 [14, 27].

The diagnosis of CHF as complication of AH was established on the basis of patients' complaints, anamnesis, physical, laboratory and instrumental research methods in accordance with the Protocol of medical care for patients with chronic heart failure approved by the Ministry of Health of Ukraine №436 from 03.03.2006 and recommendations of the Association Cardiologists of Ukraine of the Ukrainian Association of Heart Failure Specialists (2012) and Recommendations for the diagnosis and treatment of heart failure of the European Society of Cardiology (2012) [26]. The myocardial systolic function was assessed by ejection fraction. Systolic function was considered to be reduced in cases where the ejection fraction was less than 45%.

Enzyme-linked immunosorbent assay, "BIOMEDICA" reagents (Germany) and "Humareader single" strip enzyme analyzer (Germany) were used to determine the plasma concentration of CNP. Genotyping of the ET-1 gene was performed using the polymerase chain reaction. This study was conducted jointly with the Research Institute of Genetic and Immunological Foundations of Pathology and Pharmacogenetics of the Ukrainian Medical Dental Academy (Poltava, headed by Prof. I.P. Kaidashev).

Mathematical processing of the material was performed on a personal computer using the standard statistical package STATISTICA 6.0. Mathematical processing of results:

1. The calculation of primary statistics included the calculation of the arithmetic mean (M), the error of the arithmetic mean (m), the standard deviation (σ).

2. Comparison of the arithmetic means of the two samples distributed according to the law of normal distribution was evaluated by Student's t-test (t). For samples whose distribution did not comply with the law of normal distribution the differences were assessed according to the U-Mann-Whitney criteria.

In determining the limit level of CNP in blood plasma used the formula proposed by M. I. Antomonov in co-authorship with V.M. Zhebel, O.O. Sakovich, G.V. Vilchinsky,
Results

The first step was to determine the level of CNP in the blood of men in all study groups regardless of the carrier of the polymorphic variant of the ET-1 gene. Plasma CNP levels in men with AH and LVH (5.21±0.11) pmol/ml and in patients with AH and CHF (5.22±0.13) pmol/ml were significantly higher than in control patients (2.35±0.06) pmol/ml (p<0.0001), however did not differ in patients with AH. The obtained results allowed to calculate the limit level of CNP which is 3.37 pmol/ml (sensitivity - 95.57%, specificity - 83.11%, infallibility - 79.09%, false-negative response - 6.14%, false-positive response - 16.24%) and can be used for screening detection of males with persistent long-term increase in BP. Such patients will be subject to further follow-up to confirm the diagnosis and severity of AH.

It was determined that the frequency distribution of polymorphic variants of the ET-1 gene among men with AH corresponded to the Hardy-Weinberg equilibrium. It was found that in men from the control group, the Lys/Lys genotype of the ET-1 gene predominates. It was established that in men with AH as in the control group the Lys allele is dominant but no significant difference was found in the frequency of ET-1 gene alleles between the control group and among patients with AH (p>0.05).

The next step was to determine the frequency of alleles of the ET-1 gene in individuals from all groups (Tab. 2).

We also determined the levels of CNP in healthy individuals and in patients with AH carriers of polymorphic variants of the ET-1 gene (Tab. 3). It is established that in men with AH the level of CNP is significantly higher than in the control group in carriers of any polymorphic variants of the ET-1 gene. In patients with AH carriers of the Lys allele have significantly higher levels of CNP than carriers of the Lys/Lys genotype which may indirectly indicate a corresponding endothelial response to higher concentrations of vasoconstrictor ET-1 and the severity of AH.

Table 1. Frequency of ET-1 gene variants in men of the control group and in patients with AH (%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Carriers of the Lys/Lys genotype</th>
<th>Carriers of the Asn/Asn allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=79)</td>
<td>65.82% (n=52) (1)</td>
<td>34.18% (n=27) (4)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Men with AH and LVH (n=62)</td>
<td>56.45% (n=35) (2)</td>
<td>43.55% (n=27) (5)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Men with AH and CHF (n=50)</td>
<td>66.00% (n=33) (3)</td>
<td>34.00% (n=17) (6)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p</th>
<th>p_1&gt;0.05</th>
<th>p_2&gt;0.05</th>
<th>p_3&gt;0.05</th>
<th>p_4&gt;0.05</th>
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<td>p_1&gt;0.05</td>
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</table>

Table 2. Frequency of alleles of the ET-1 gene in men of the control group and in patients with AH (%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Carriers of the Lys allele</th>
<th>Carriers of the Asn allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=79)</td>
<td>79.75% (1)</td>
<td>20.25% (4)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Men with AH and LVH (n=62)</td>
<td>73.39% (2)</td>
<td>26.61% (5)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Men with AH and CHF (n=50)</td>
<td>80.00% (3)</td>
<td>20.00% (6)</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p</th>
<th>p_1&gt;0.05</th>
<th>p_2&gt;0.05</th>
<th>p_3&gt;0.05</th>
<th>p_4&gt;0.05</th>
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<td></td>
<td>p_1&gt;0.05</td>
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O. O. Singh [1, 28].

X=([M1+2 • m1]+(M2-2 • m2))/2,

where X is the limit level of the peptide;

M1 - the average value of the peptide level in the group with no symptoms (relatively healthy);

m1 - error M1;

M2 - the average value of the peptide level in the group with the presence of the sign (conditionally ill);

m2 - error M2.

Sensitivity, specificity, infallibility, false-negative and false-positive responses of the proposed methods were established when determining the threshold level and during the discriminant analysis [10]:

Sensitivity = (100 • a)/(a+b) (%),

where a - the number of conditionally ill persons with the presence of the symptom;

b - the number of conditionally ill persons with no symptoms.

Specificity = (100 • d)/(c+d) (%),

d - the number of relatively healthy persons with no symptoms.

Infallibility = 100 • (a+d)/(a+b+c+d), (%).

False-negative response = (100 • b)/(a+b), (%).

False-positive response = (100 • c)/(c+d), (%).
Table 3. Plasma concentration of CNP in men of the control group and with AH, carrying different variants of the ET-1 gene, pmol/ml (M±m).

<table>
<thead>
<tr>
<th>Group</th>
<th>Carriers of the Lys/Lys genotype</th>
<th>Carriers of the Asn allele</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=79)</td>
<td>2.02±0.29 (n=52) (1)</td>
<td>2.98±0.08 (n=27) (4)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Men with AH and LVH (n=62)</td>
<td>4.68±0.12 (n=35) (2)</td>
<td>5.90±0.11 (n=27) (5)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Men with AH and CHF (n=50)</td>
<td>4.88±0.09 (n=33) (3)</td>
<td>5.93±0.18 (n=17) (6)</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion

It was revealed that the plasma levels of CNP in men with AH are significantly higher than in control patients, however do not differ in patients with AH from different groups. Similar studies were conducted by the staff of the Department of Internal Medicine of the Medical Faculty №2 Vinnytsya National Pirogov Memorial Medical University [6, 21, 23], however in this paper for the first time it was calculated the limit plasma level of CNP in men for screening of individuals with AH who has persistent long-term elevation of BP and will require further examination to clarify the diagnosis.

The obtained data on the carrier frequencies of individual variants of the ET-1 genotype are consistent with the results of researchers in Kazakhstan, Hungary and Sumy (Ukraine) [2, 6, 17]. A. E. Gaipov, and A. U. Dzholdasbekova (2010) showed that the Lys/Lys genotype in men with AH is 1.3 times less than in the control group, however in both healthy individuals and patients with AH it is dominant. The Lys/Asn genotype occurs with equal frequency in two groups. The polymorphic variant of Asn/Asn was determined only in patients with hypertension. Also, the Lys allele was predominant in men with AH and in the control group [7]. The results obtained by the residents of Sumy differ slightly from the above. In general the Lys/Lys genotype of the ET-1 gene dominates in both men and women in both the control group and those with ischemic stroke. In the control group of women, the Lys/Lys genotype significantly dominates and in the group of patients with ischemic stroke Asn allele carriers (Lys/Asn and Asn/Asn genotypes) predominate. However, in men such a difference between the study groups was not found. The Lys/Lys and Asn/Asn genotype was significantly more common in men with ischemic stroke and Lys/Asn in women [16]. In J. J. Jin et al. (2003) (Japan) the frequency of ET-1 gene variants in men and women with AH looks like this - genotype Lys/Lys - 53%, Lys/Asn - 38%, Asn/Asn - 8%, Lys allele - 72%, Asn - 28% [9]. A Hungarian study of adolescents of different sexes with AH showed that the Lys/Lys, Lys/Asn and Lys alleles were probably more common than the Asn/Asn genotype and the Asn allele but there was no difference between the Lys/Lys and Lys/Asn genotypes. It should be noted that the Lys/Lys and Asn/Asn genotypes were more often found in the control group the Lys/Asn genotype - in patients with AH [2].

In patients with AH - carriers of the Lys/Lys genotype and Asn allele of the ET-1 gene - the levels of CNP in blood were higher than in the control group - carriers of similar variants of the ET-1 gene. A study of the level of CNP in the plasma of the control group and in patients with AH, showed that it is in carriers of the Asn allele level is probably higher than in carriers of the Lys/Lys genotype as a likely response to increased vasoconstrictors in blood at the heart of hypertension.

Conclusions and prospects for further development

1. It is determined plasma limit level of CNP for screening detection of patients with persistent long-term increase in blood pressure on the background of hypertension.

2. In men living in the Podillia region of Ukraine aged 40-60 years the polymorphism of the ET-1 gene is manifested by the dominance of the Lys/Lys genotype and the Lys allele. In individuals without cardiovascular disease and in patients with hypertension the carrier of the Asn allele of the ET-1 gene is associated with higher levels of C-natriuretic peptide compared to Lys/Lys homozygotes. Carriers of the Asn allele can be used to identify groups of patients who require more intensive treatment or more frequent observation by a cardiologist. No similar studies have been found in Ukrainian and foreign literature.

References


С-натрійуретичний пептид - як показник стійкого підвищення артеріального тиску у чоловіків з гіпертонічною хворобою

Палагнюк Г. О., Пашкова Ю. П., Матохнюк М. О., Франчук С. В., Жебель В. М.

Анотація. За сучасними уявленнями гіпертонічна хвороба (ГХ) є багатофакторним захворюванням. Визнано, що одним із ключових механізмів стійкого підвищення артеріального тиску (АТ) є порушення балансу між вазоконстрикцією та вазодилатацією судин, що зумовлено дисфункцією ЕТ-1. У цьому відношенні, маловивченими залишаються взаємозв'язки стабільних плазмових змін плазмового рівня СНП у групах з ГХ з різним генотипом поліморфних варіантів гена ЕТ-1. У дослідженні прийняв участь 191 чоловік віком 40-60 років, із них 79 осіб склали контрольну групу, 62 особи з ГХ ІІ стадії, 50 пацієнтів з ГХ ІІІ стадії (що ускладнена ХСН ІІ А стадії І І-ІІІ ФК за NYHA). Генотипування поліморфних варіантів гена ЕТ-1 проведено із застосуванням полімеразної ланцюгової реакції. Для визначення плазмової концентрації СНП рівень стандартного статистичного пакету STATISTICA 6,0. Встановлено, що плазмові рівні СНП у чоловіків з ГХ ІІ стадії (5,21±0,11) пмоль/мл та у пацієнтів з ГХ ІІІ стадії (5,22±0,13) пмоль/мл є достовірно вищими, ніж у пацієнтів контрольної групи, однак, ніяких достовірних різниць між групами в результаті генотипування поліморфних варіантів гена ЕТ-1 не виявлено (р>0,05). Слід відмітити, що у пацієнтів з ГХ різної стадії, при носійстві усієї палеї поліморфних варіантів гена ЕТ-1, рівень СНП в плазмі крові також виявився достовірно вищим, ніж у контрольній групі, однак, саме у групи Аспл плазмовий рівень СНП нижчий, ніж у групі з генотипом Lys/Lys у всіх групах дослідження. Таким чином, досліджено, що середня плазмова концентрація СНП у групах з ГХ ІІ та ІІІ стадій нижча, ніж у групі контролю, що дало можливість встановити межовий рівень пептиду для скринінгового виявлення осіб із стійким довготривалим підвищенням АТ. У чоловіків групи контролю та у пацієнтів з ГХ ІІ та ІІІ стадій насамперед аспл ЕТ-1 асоціюється з вищим рівнем СНП, проте саме в усіх групах дослідження домінантним був генотип Lys/Lys та аспл ЕТ-1-генна ІІ стадії.

Ключові слова: С-натрійуретичний пептид, гіпертонічна хвороба, хронічна серцева недостатність, поліморфізм гена ендотелін-1.