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The BNP genepolymorphism in women and plasmapeptide levels for the screening diagnostics of chronic heart failure in essential hypertension

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Abstract— Essential hypertension (EH) and its complication, a chronic heart failure (CHF), are one of the most common pathological tandems in practical cardiology. Link edetiologically and pathogenetically, they share a common path of progression, manifested by structural, functional, and humoral disturbances in cardiovascular homeostasis. The mainstructural changes are an increase in the mass, volume and shape of the left ventricle(LV) due to hypertrophy of cardiomyocytes, hypertrophy and hyperplasia of the interstitium and endothelial cells. Left ventricular hypertrophy is a clear clinical marker of EH progression and deepening of pathological processes in the cardiovascular system. In recent years, a member of the family of natriuretic peptides, brain natriuretic peptide (BNP), has taken the place of one of the biomarkers of myocardial and vascular remodeling processes, most often used in practical medicine; it is also positioned as a humoral antagonist of the activity of renin-angiotensin-aldosterone system(RAAS), which is the leading pathogenetic link in most cases of hypertensive cascade and CHF. However, it is generally known that plasma levels of the peptide depend on numerous factors: the presence of certain cardiovascular pathology, sex, age, comorbidity, and genetic background. This studyaims to supplement the data on the use of BNP as a signaling indicator of changes in myocardial structure and function and calculate the screening threshold levels of peptide in uncomplicated and complicated EH in postmenopausal women with polymorphic variants of the BNP gene. 180 women aged 40-64 living in the Podilliaregion of Ukraine were examined: 67 women without signs of cardiovascular pathology, who were included in the control group, 62 women with uncomplicated EH with LVH,and 51 women with EH complicated by CHF. All the patients were examined according to a standardized plan: general clinical, instrumental, and laboratory examination was conducted. The genotyping of the BNP gene was performed using the polymerase chain reaction. Plasma concentrations of natriuretic peptide were determined by enzyme-linked immunosorbent assay. It was revealed that the frequency of carrying the T381C genotype and the C allele significantly prevails among the polymorphic variants of the BNP gene in women 40-65 years of age. The highest level of plasma BNP concentration, which was 193,27 ± 2,98 pg/ml, was determined in women with EH complicated by CHF.In addition, carrying the C alleleof the BNP gene is significantly associated with higher levels of peptide in the blood plasma of women in all the examined groups. In women 40-65 years of age with EH, the value of BMI is inversely correlated with the level of BNP in blood plasma.

Keywords. Biomarker, BNP, gene, alleles, plasma levels, essential hypertension, chronic heart failure, remodeling, left ventricular hypertrophy.

1. Introduction.

Essential hypertension (EH) occupies a leading place in the hierarchy of circulatory system diseases and has long exceeded the scope of a purely medical problemhaving become a significant social and economic burden [29]. Older age, positive family history, male gender, smoking, overweight, atherosclerosis, stress, and hypodynamia– to name a few –are major risk factors for EH, which determine its multifactorial nature [9,11,12,13,28].

For a long time, an increase in blood pressure remains the only leading objective clinical manifestation of EH. Over time, with increasing duration of hypertensive history and progression of EH, cardiomyocytes and blood vessels begin functioning under conditions of constant increased load, which ultimately leads to their restructuring.

On the one hand, myocardial remodeling is a compensatory reaction that ensures the functional capacity of the heart in conditions of constantly elevated blood pressure, on the other,itisone of the natural stages of progression of pathological changes in the heart muscle. Cardiomyocyte rearrangement processes result in different types of left ventricular hypertrophy (LVH). These myocardial changes are closely associated with a high risk of developing complications such asheart rhythm disorders, myocardial infarction (MI), cerebrovascular accidents, and chronic heart failure (CHF).

CHF is one of the leading complications of EH. Hence, EH and CHF should be viewed not as separate nosologies, but exclusively as pathologies related etiologically and pathogenetically (RAAS activation, insulin resistance, lipid metabolism disorders, etc.), as well as such that have a very natural course of development and progressioninfluenced by genetic, humoral, sexual, and population characteristics and manifested over time by deepening myocardium and blood vessels remodelingprocesses [24].

Regulation of normal blood pressure is a multilevel system. In the pathogenesis of EH, a central role belongs to the imbalance between factors contributing to vasospasm and blood pressure increase, the so-called pressorfactors, and those contributing to vasodilation, i.e. depressant factors. The first group includes the sympathoadrenal system, RAAS, ET-1, vasopressin, prolactin, adrenocorticotropic hormone, thromboxane A_2, prostaglandins of groups A and F while the effects of the second group are provided by the system of natriuretic peptides (NUP), bradykinin, prostacyclins, prostaglandins of groups E and I,etc. [11,12,13,28,29].

Such a complex cascade mechanism of BP control involves the presence of a massive genetic basis that ensures its functioning [12,25].

The list of the above risk factors for the development of EH can be extended by adding specific features of inheriting polymorphic variants of genes that encode substances such as natriuretic peptides, which are considered to be involved in the regulation of RAAS activity.

One of the most extensively studied single-nucleotide polymorphisms (SNPs) concerningits influence on the structural and functional state of the cardiovascular system is rs198389 (T381C) single-nucleotide polymorphism of the BNP gene, whose expression determines the synthesis, functioning and biodegradation of natriuretic peptides (NUP)[2, 5,12,18,25].

Several researchers point out that polymorphism of the BNP gene may be another etiological factor in fluctuations in BNP plasma concentration in situations where scientists rely on its biomarker values (differential diagnosis of dyspnea in CHF, diagnostics in certain clinical cases of LVH, progression of CHF in EH, etc.) [3,12,15,16,17,25].

It is well known that sexual dimorphism may be an additional determining factor in these processes,as early works [21] indicate the importance of considering gender in researching biochemical manifestations of inheriting the polymorphismof the same gene in men and women.

In most studies on the BNP biomarker, sexually mixed groups of patients with EH were researched [4,6,10,14,19,20,22,23,26,27]. Whilethese processes were investigated earlier in the male population of the Podilliaregion of Ukraine[16,17], no such studies were conducted on Ukrainian women.

This study is the first to determine the limits of the BNP levels taking into account the inheritance of polymorphic variants of the BNP gene in women of postmenopausal age of the Podillia region of Ukraine with uncomplicated EH and EH complicated by CHF.

Aim.
To improve and personify the diagnostics of LVH in uncomplicated EH and predict CHF by determining BNP levels in blood plasma taking into account the carrying of polymorphic variants of the BNP gene in postmenopausal women aged 40-65 yearsliving in the Podillia region of Ukraine.

2. Materials and research methods.

According to the study design, 180 postmenopausal women (40-65 years old) living in the Podillia region of Ukraine for three consecutive generations were examined. 67 people without signs of cardiovascular pathology formed a control group. The general cohort was divided into two groups: the first consisted of women with uncomplicated EH with left ventricular hypertrophy (LVH) with preserved systolic function (62 people), the second comprised patients with EH complicated by CHF not higher than class II-III according to NYHA (51 people). The mean age of women with uncomplicated EH was 57,3 ± 0,61 years, the mean age of women with EH complicated by CHF was 59,9 ± 0,59 years, the control group comprised women of 56,43 ± 0,64 years.

All data obtained from the examination of this population of women were compared with the results of examining the groups of men of the same age and clinical status. The information about 191 males was borrowed from the original scientific study in agreement with the author (I.P. Pashkova) [16,17].

Detailed information about the women constituting the control group was highlighted in a previous publication by the author [21]. In this article, the results are presented for comparison.

Females were only included in the study based on the presence of anamnestic indication for menopause (absence of menstruation for 6 months or more) and subsequent confirmation of this involutive state by laboratory determination of the concentration of follicle-stimulating hormone (FSH) in blood plasma.

The selection of individuals for the study was conductedby interviewing, detailed history taking, general clinical, instrumental and laboratory examination, and retrospective analysis of medical records following the principles of the World Medical Association (WMA) Declaration of Helsinki and after signing informed consent to participate in research [7].

Severe cardiac arrhythmias, bronchial asthma or chronic obstructive pulmonary disease, severe renal and hepatic impairment, endocrine disorders, circulatory diseases, rheumatic, autoimmune diseases, and neoplasms were identifiedas exception criteria.

The diagnosis of essential hypertension was established according to the clinical recommendations of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) in 2018. The class of CHF was determined based on the criteria of the New York Heart Association.

Polymorphic variants of the BNP gene (SNP rs198389: T381C) were identified by polymerase chain reaction (PCR). The study was conducted jointly with the Research Institute of Genetic and Immunological Foundations of Pathology and Pharmacogenetics of a Higher State Educational Institution of Ukraine"Poltava State Medical University" (Poltava, head – Prof. I.P.Kaidashev) [25].

The concentration of B-natriuretic peptide (BNP) in the blood plasma of the subjects was determined by ELISA (enzyme-linked immuno sorbent assay). The set of reagents "Elabscience® Human BNP (Brain Natriuretic Peptide) ELISA Kit" (USA) was used.

Mathematical processing was performed on a personal computer using the standard statistical package Statistica 10.0. The frequency of distribution of polymorphic gene variants in the population was checked according tothe Hardy-Weinberg equilibrium [8].

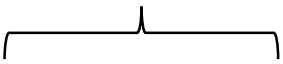
3. Results and discussion.

The cytogenetic study on carrying polymorphic variants of the BNP gene (SNP rs198389: T381C) revealed that among postmenopausal women of the Podillia region of Ukraine, constitutingthe control group, 31,34% (n = 21) of women have a variant of the genotype T381T, 52,24 % (n = 35) of women – the genotype T381C, and 16,42% (n = 11) are homozygous for the C allele, i.e. they have the genotype C381C.

In individuals with uncomplicated EH with LVH, the genotype T381T was determined in 35,48% (n = 22) of the subjects while in 50,00% (n = 31) of the examined, the genotype T381C was identified and 14,52% (n = 9) of women were homozygous for C381C.

In women with EH complicated by CHF, the genotype T381T was detected in 37,25% (n = 19) of the subjects, the genotype T381C – in 47,06% (n = 24), and the genotype C381C – in 15.69% (n = 8) of the respondents. Therefore, in women with EH, both complicated and uncomplicated, the genotype T381C significantly prevails ($p < 0.05$).

The frequency distribution of polymorphic variants of the BNP gene in groups of males did not differ from that in groups of females. Also, in men of all the surveyed groups, a significant predominance of the genotype T381C was established (Fig. 1).



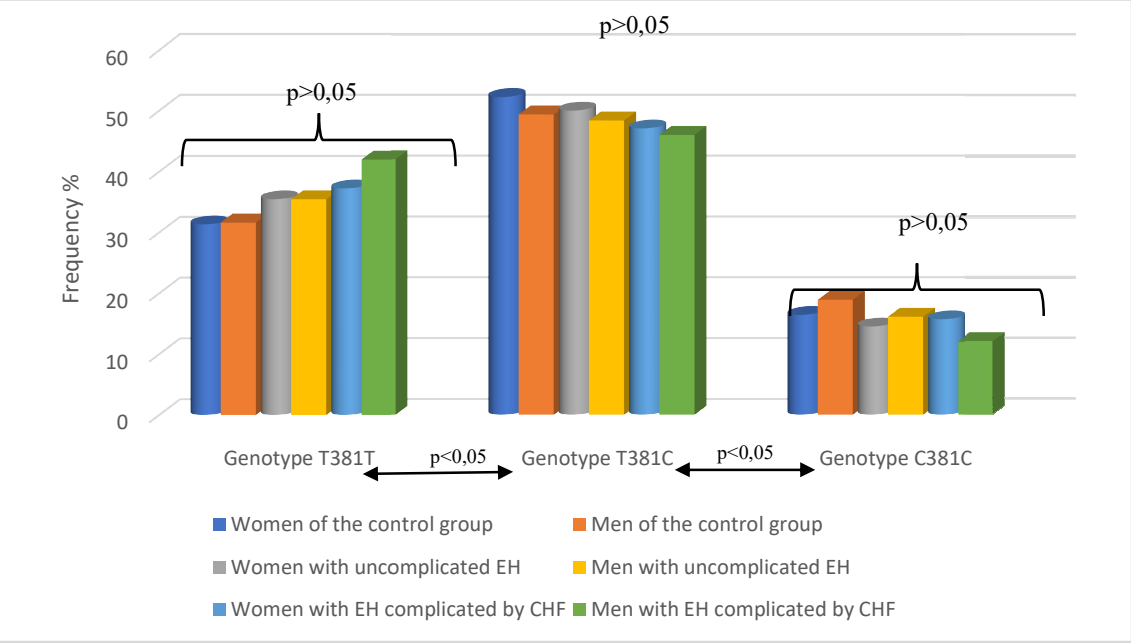


Fig. 1. Distribution of frequencies of inheriting polymorphic variants of the BNP gene among the residents of the Podillia region of Ukraine with uncomplicated EH and EH complicated by CHF (%)

For unification and convenience of the study results analysis, heterozygotes T381C and homozygotes C381C were combined into a group of the C allele carriers.

It was found that in the groups of women, in both uncomplicated EH and EH complicated by CHF, the C allele carriers significantly predominate. Similar results were obtained in the study of the corresponding groups of men.However, no significant difference was revealed in the data between men'sand women'sgroups (Fig. 2).

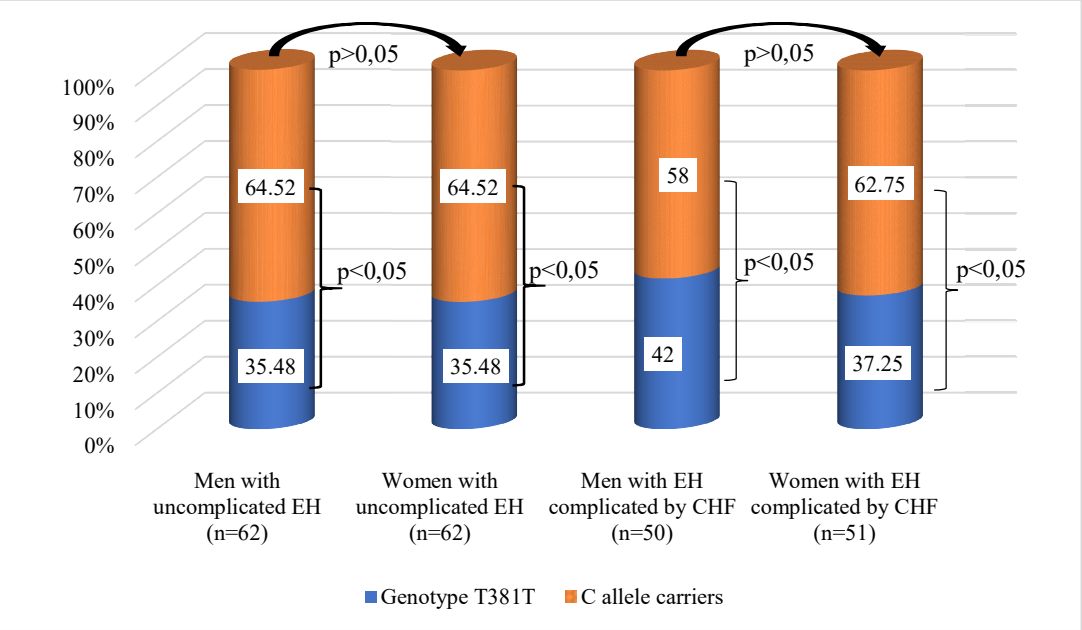


Fig. 2. Distribution of frequencies of inheriting the genotype T381T and the C allele among the residents of the Podillia region of Ukraine with uncomplicated EH and EH complicated by CHF (%).

The described results confirm the data obtained by examining mixed populations in the United States, Germany, and Russia [2,5,18]. The published materials indicated a significant predominance of the genotype T381C and the C allele in the cohorts of both men and womenregardless of ethnic identity or territorial affiliation.

Homeostasis and cardiovascular system functioning result from a complex interaction of various regulatory factors. BNP is one of the instrumental biomarkers that signals changes in these indicators,being one of the components of a multilevel structure that opposes RAAS. Determination of the plasma concentration of the peptide is a proven early and subtle indicator of these changes.

Thus, it was found that the BNP level in the blood plasma of women with uncomplicated EH and LVH is 82,15 ± 1,47 pg/mlwhile in women with EH complicated by CHF, it equals 193,27 ± 2,98 pg/ml and is significantly higher than the indicators in the control group and similar male groups (p <0,05) (Fig. 3).

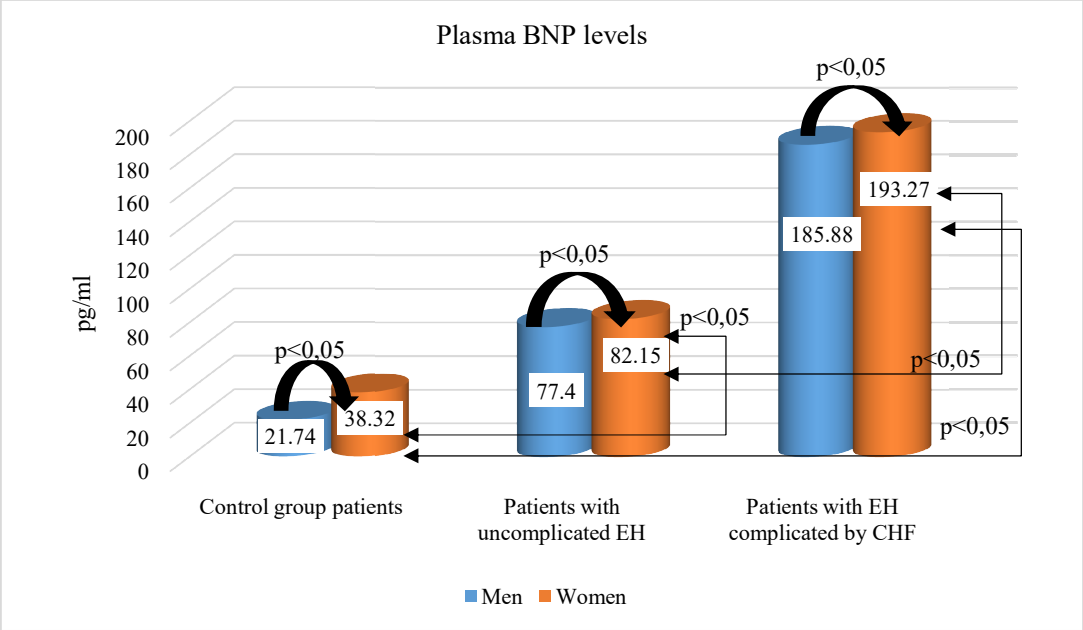


Fig. 3. Plasma BNP levels in the residents of the Podillia region of Ukraine, pg/ml.

Determination of plasma BNP levels taking into account polymorphic variants of the BNP gene carrying revealed that both homozygotes T381T and the C allele carriers have the highest BNP indicators in case of EH complicated by CHF. At the same time, in all the groups of the examined women, the C allele carriers had significantly higher levels of peptide in blood plasma compared with homozygotes T381T (p <0,05) (Table 1).

Table 1 Plasma BNP levels in women of the main group in case of inheriting polymorphic variants of the BNP gene, pg/ml

Group	Control	Patients with	Patients with EH	p

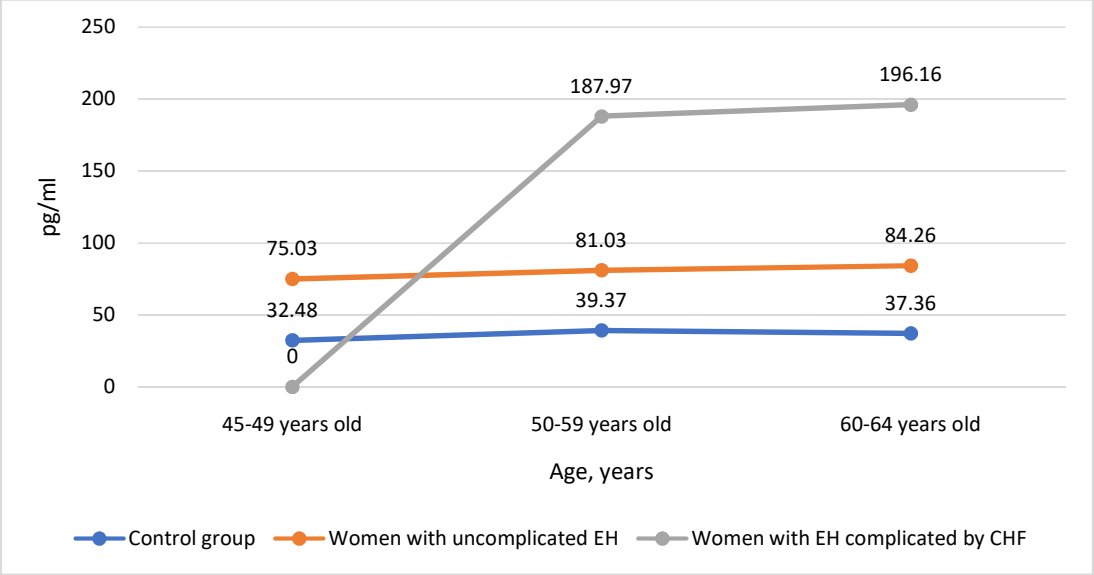
Genotype	group (n=67)	uncomplicated EH (n=62)	complicated by CHF (n=51)	
HomozygotesT381T (n=62)	34,6 ± 1,28 (n=21) (1)	78,20 ± 2,46 (n=22) (2)	182,63 ± 2,74 (n=19) (3)	p_{2-1}<0,05 p_{3-1}<0,05 p_{3-2}<0,05
C allele carriers (n=118)	40,03 ± 0,61 (n=46) (4)	84,32± 1,77 (n=40) (5)	199,59 ± 4,10 (n=32) (6)	p_{5-4}<0,05 p_{6-4}<0,05 p_{6-5}<0,05
p	p_{4-1}<0,05	p_{5-2}<0,05	p_{6-3}<0,05	

Over many years, BNP has rightly occupied a prominent place of a "universal biomarker." According to the recommendations of the European Society of Cardiology (ESC, 2019), the plasma level of BNP ≤ 35 pg/ml indicates a stable course of CHF, and with the development of acute heart failure, it reaches ≥ 100 pg/ml [15]. However, the results obtained in the Podillia population of women with EH prompted the revision of the average plasma concentrations of the peptide and the calculation of threshold levels of BNP to improve and personify the diagnostics of changes in the cardiovascular system. They can become screening "cut-off points" for the diagnostics of EH with LVH and CHF, taking into account the carrying of the genotype T381T or the C allele of the BNP gene.

It was found that:
–BNP level ≥ 55,44 pg/ml (sensitivity – 95,4%, specificity – 92,00%, infallibility – 96,20%, false-negative response – 4,6%, false-positive response – 8,00%) allows diagnosing LVH in uncomplicated EH in female homozygotes T381T;
–BNP level ≥ 130,135 pg/ml (sensitivity –98,00%, specificity–95,5%, infallibility –98,00%, false-negative response – 2,00%, false-positive response–4,5%) allows diagnosing EH complicated by CHF in female homozygotes T381T;
–BNP level ≥ 61,015 pg/ml (sensitivity – 92,00%, specificity – 90,70%, infallibility – 84,14%, false-negative response –8,00%, false-positive response – 9,30%) allows diagnosing LVH in uncomplicated EH in female C allele carriers;
–BNP level ≥ 139,625 pg/ml (sensitivity – 98,8%, specificity –98,24%, infallibility–92,00%, false-negative response – 1,2%, false-positive response – 1,76%) allows diagnosing EH complicated by CHF in female C allele carriers.

Apart from the associations between peptide plasma levels and the expression of polymorphic variants of the BNP gene, the study also investigated its possible relationships with age and body weight.

Statistical analysis revealed that women over 60 years old with EH complicated by CHF have significantly higher BNP levels in blood plasma than younger patients and women without CHF (p <0,05) (Fig. 4).



*the study disregarded 45-49-year-oldwomensufferingfromEHcomplicatedbyCHF

Fig. 4. Plasma BNP levels in women of the main group depending on age, pg/ml

Numerous local and foreign sources mention an inverse relationship between BMI and plasma levels of natriuretic peptides [1,22]. The results of this study confirmed this pattern. In women of the main group, the highest indicators of plasma BNP levels were verified in women with normal body weight, and they were lower with higher BMI ($p<0,05$). In men, similar data were determined only for individuals with EH complicated by CHF (Table 2).

Table 2 Plasma BNP levels depending on BMI and sex, the main group

EH \ BMI		Norm 18,5-24,9	Excessweight 25-29,9	Obesity 30,0 andhigher	p
Uncomplicated EH	Women (n=62)	97,85± 2,26 (n=10) (1)	86,65± 0,89 (n=23) (2)	73,16± 1,60 (n=29) (3)	$p_{2-1}<0,05$ $p_{3-1}<0,05$ $p_{3-2}<0,05$
	Men (n=62)	78,46 ± 4,11 (n=28) (4)	76,99 ± 5,07 (n=21) (5)	75,79 ± 6,73(n=13) (6)	$p_{5-4}>0,05$ $p_{6-4}>0,05$ $p_{6-5}>0,05$
EH complicatedby CHF	Women (n=51)	237,42 ± 10,85 (n=6) (7)	197,48 ± 1,01 (n=18) (8)	180,66 ± 1,89 (n=27) (9)	$p_{8-7}<0,05$ $p_{9-7}<0,05$ $p_{9-8}<0,05$
	Men (n=50)	197,52 ± 7,94 (n=4) (10)	179,38 ± 8,97 (n=21) (11)	158,08 ± 7,50 (n=25) (12)	$p_{11-10}<0,05$ $p_{12-10}<0,05$ $p_{12-11}<0,05$
p		$p_{4-1}<0,05$ $p_{10-7}<0,05$	$p_{5-2}<0,05$ $p_{11-8}<0,05$	$p_{6-3}<0,05$ $p_{12-9}<0,05$	

To individualize the diagnostics of EH and CHF in practical medicine, with different phenotypic features of metabolism, it was proposed to use threshold levels of plasma BNP concentration for women with different BMI values.

With a BMI of 18,5-24,9:

– BNP level ≥ 69,1 pg/ml allows diagnosing EH with LVH;
– BNP level ≥ 159,045 pg/ml allows diagnosing EH complicated by CHF.

With a BMI of 25-29,9:

– BNP level ≥ 61,38 pg/ml allows diagnosing EH with LVH;
– BNP level ≥ 141,945 pg/ml allows diagnosing EH complicated by CHF.

With a BMI of 30,0 and above:

– BNP level ≥ 50,825 pg/ml allows diagnosing EH with LVH;
– BNP level ≥ 126.62 pg/ml allows diagnosing EH complicated by CHF.

In this case, for all the calculated indicators, sensitivity equals 100.00%, specificity – 100.00%, infallibility – 100.00%, false-negative answer – 0.00%, false-positive answer – 0.00%. No such calculations were performed for men.

While examining the currently accepted BNP thresholds it is necessary totake into account the fact that NUP levels may be persistently elevated in chronic heart failure, which precludes their representativeness in acute hemodynamic changes, and may differ in patients of different sexes and body weights. Knowledge of the individual concentration of BNP in each patient with a stable clinical status (the so-called dry matter concentration of NUP), will help to interpret its indicators in acute cardiovascular events and decompensation [15].

It is quite obvious that the differences in results between female and male cohorts require a further in-depth study of all relevant factors influencing the development of thestructural and functional disorders of the cardiovascular system and the manifestation of humoral factors signaling these changes. In addition, future research should be conducted based on different populations (ethnic, sexual, territorial), involving more subjects and using new promising methods.

4. Conclusions.

1. In the population of women of 40-65 years of age, living in the Podillia region of Ukraine, the genotype T381C and C allele carrying is registered more frequently.
2. Higher plasma BNP levels are determined in women with EH complicated by CHF, compared with the control group and patients with uncomplicated EH.
3. Carrying the C allele of the BNP gene is associated with higher concentrations of BNP in the blood plasma in all the examined groups of women.
4. Older age is one of the determining factors for peptide plasma levels: women over 60 years of age suffering from EH complicated by CHF had the highest BNP indicators in blood plasma.
5. It was established that in the main group of women, higher BMI values were significantly associated with lower plasma levels of BNP.

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