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Gene Polymorphism of ET-1 and Its Plasma Levels in Men with Uncomplicated Essential Hypertension and Left Ventricular Hypertrophy

Hanna O. Palahniuk

Vinnytsia National Pirogov Memorial Medical University
Department of Internal Medicine of the Medical Faculty №2
56 Pirogov Str., Vinnytsia UA-21018, Ukraine

Address for correspondence:

38A/30 Skaletskyy Str., Vinnytsia UA-21000, Ukraine

Iuliia P. Pashkova

Vinnytsia National Pirogov Memorial Medical University
Department of Internal Medicine of the Medical Faculty №2
56 Pirogov Str., Vinnytsia UA-21018, Ukraine

Address for correspondence:

65/2 L.Ukrainka Str., Vinnytsia UA-21020, Ukraine

Vadym M. Zhebel

Vinnytsia National Pirogov Memorial Medical University
Department of Internal Medicine of the Medical Faculty №2
56 Pirogov Str., Vinnytsia UA-21018, Ukraine

Address for correspondence:

Department of Internal Medicine of the Medical Faculty №2 Vinnytsia National
Pirogov Memorial Medical University 35/19 V. Porik Str., Vinnytsia UA-21030,
Ukraine

Olena O. Sakovych

Vinnytsia National Pirogov Memorial Medical University
Department of Internal Medicine of the Medical Faculty №2
56 Pirogov Str., Vinnytsia UA-21018, Ukraine

Address for correspondence:
50/144 40th Anniversary of the Victory Str., Vinnytsia UA-21036, Ukraine

Natalia V. Zhebel
Vinnytsia National Pirogov Memorial Medical University
Department of Internal Medicine of the Medical Faculty №2
56 Pirogov Str., Vinnytsia UA-21018, Ukraine

Address for correspondence:
35/19 V. Porik Str., Vinnytsia UA-21030, Ukraine

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Abstract

Aim: To improve diagnosis of the severity of essential hypertension in men citizens of Podillia region in Ukraine by determining the plasma levels of ET-1 in patients with different ET-1 gene variants.

Methods. We examined 141 men aged 40 – 60 years old, who live in Podillia region in Ukraine. Among them 62 men were diagnosed uncomplicated EH with left ventricular hypertrophy and CHF I classe according to NYHA Classification. 79 healthy men were included into a control group. The patients with uncomplicated EH and the healthy men were representative by age. The ET-1 (Lys198Asn) gene polymorphism was determined by PCR, and the level of ET-1 plasma concentrations was established by ELISA.

Results. In both healthy men and patients with uncomplicated EH with LVH, residents of Podillia region in Ukraine aged 40-60 years old, dominates Lys198Lys genotype and Lys allele of the ET-1 gene. It was found that any inherited variant of the ET-1 gene was not associated with the risk of developing uncomplicated EH with LVH in men residents of Podillia region in Ukraine. However, the carriers of the Asn allele of the ET-1 gene have significantly higher levels of ET-1 in plasma in both healthy men and patients with uncomplicated EH and LVH. There are calculated levels of ET-1 that can be used for screening of large groups of people for early diagnosis of uncomplicated EH with LVH in carriers of the Asn allele and carriers of the genotype Lys198Lys of the ET-1 gene.

Keywords: essential hypertension, gene polymorphism of endothelin-1, plasma concentrations of endothelin-1.

Introduction

Essential hypertension (EH) affects about 25% of the adult population in

the world, and 7.6 million deaths (about 13.5% of the total deaths), 54% of strokes and 47% of events caused by coronary heart disease are the result of high blood pressure (BP) [16]. It is well known that EH is a polygenic and multifactorial disease which development depends on genetic predisposition and metabolic imbalance in the human body and environmental factors [9].

Many clinical and experimental works are being published about the role of endothelial dysfunction (ED) in the occurrence and progression of EH [15,24]. It is believed that endothelin-1 (ET-1) plays a key role in the pathogenesis of ED [2,13] and its plasma concentration increases in the early stages of EH [11,21,22]. It is found that ET-1 is one of the most powerful vasoconstrictor. Synthesis of ET-1 is stimulated by thrombin, catecholamines, angiotensin, vasopressin, insulin, interleukins, cell growth factor, thromboxane A₂, oxidized lipoproteins of low and high density, hypoxia, ischemia, mechanical irritation and others. ET-1 inhibitors are natriuretic peptide, endothelin-3, prostaglandins E₂ and I₂ (prostacyclin), nitric oxide [1]. It is proved that there is a connection between ET-1 in plasma and variants of left ventricular hypertrophy (LVH) and also the change of its plasma concentration in presence or absence of left ventricular systolic dysfunction as a result of EH [17,21,25].

Scientists are studying candidate genes that are involved in the implementation of EH and regulation of BP, one of which is the ET-1 gene. The most investigated Single nucleotide polymorphism (SNP) of the ET-1 gene is based on the replacement of the aminoacid lysine (Lys) on asparagine (Asn) at position 198 of the polypeptide chain (also known as substitution G → T) in the formation and development of EH and its complications [6,7,19,27].

However, the data about the plasma level of ET-1 in different variants of the ET-1 gene polymorphism in patients with uncomplicated EH with LVH is not numerical and has not been studied in the Ukrainian population yet at all. **The aim** of the study is to determine the plasma concentration of ET-1 in different variants of the ET-1 gene polymorphism in patients with uncomplicated EH with LVH citizens of Podillia region in Ukraine.

Methods

The study involved 141 middle-aged male residents in Podillia region in Ukraine. Among them 62 men from the main group were diagnosed uncomplicated EH with LVH with saved systolic function and chronic heart failure (CHF) I classes according to NYHA Classification, whose average age was 49,19±0,66 years old and 79 healthy men whose age (49,01±0,73 years old) did not differ from patients with uncomplicated EH with LVH and made the control group (p>0,05). The diagnosis of EH was established on the basis of the patients' complaints, anamnesis, physical examination, laboratory and instrumental methods of investigation according to the guideline of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) in 2013 [8]. Systolic function of left ventricle was assessed in terms of ejection fraction (EF).

Systolic function was considered saved when EF was over 45%. All patients during the examination were treated at Vinnytsia regional specialized dispensary of radiation protection of the Ministry of Health of Ukraine, Military Medical Center of the Central Region of Air Force of Ukraine and were observed from December 2013 to June 2014.

Exclusion criteria of the study were: secondary hypertension, renal and liver dysfunction, coronary heart disease the onset of which was before EH, endocrine, hematological, neoplastic and autoimmune disorders, patients with EH complications: myocardial infarction, acute cerebrovascular accident. Genotyping of the ET-1 gene was conducted using polymerase chain reaction (PCR) after isolation of genomic DNA from white blood cells of venous blood. This study was carried out jointly with the Research Institute of the genetic and immunological bases of pathology and pharmacokinetics "Ukrainian Medical Stomatological Academy" (Poltava, the head is prof. I.P. Kaidashev). The ET-1 concentration in plasma was determined by using ELISA method on enzyme-linked immunosorbent analyzer "Humareader single» (Germany) at 450 nm and differential filter 630 nm. To determine the ET-1 plasma concentration a standard set of the firm «DRG» (USA) was used. The mathematical processing was performed on a personal computer using a standard statistical package STATISTICA 6,0. The frequency distribution of gene polymorphisms in the population was undergone a checking according to Hardy-Weinberg equilibrium law using a calculator gene expert to calculate the number of statistical parameters in the study "case-control" which using SNP (State Scientific Center of the Russian Federation "HosNYY genetics", gen-exp.ru). Boundary ET-1 level was determined by the method proposed by M.U. Antamonov in collaboration with V.M. Zhebel, O.O. Sakovych, G.V. Wilczynskyy, O.O. Singh [3,14,32].

Results

It was established that Lys198Lys genotype of the ET-1 gene was 65,82% (n=52), Lys198Asn genotype – 27,85% (n=22) and Asn198Asn genotype - 6,33% (n=5) ($p_{Lys/Asn-Lys/Lys} < 0,00001$; $p_{Asn/Asn-Lys/Lys} < 0,00001$; $p_{Asn/Asn-Lys/Asn} < 0,001$). The frequency of the Lys allele in male from the control group was 79,75%, the Asn allele - 20,25% ($p_{Lys-Asn} < 0,00001$). Because of the low frequency of carriers of Asn198Asn genotype men with Lys198Asn and Asn198Asn genotypes from the control group were united as carriers of the Asn allele. The frequency of the Asn allele in the male control group is 34,18% (n=27) ($p_{Asn\ allele-Lys/Lys} < 0,00001$).

It was investigated that Lys198Lys genotype of ET-1 dominates in patients from the main group - 56,45% (n=35), Lys198Asn genotype was in 33,87% (n=21) patients and Asn198Asn genotype - 9,68% (n=6) ($p_{Lys/Asn-Lys/Lys} < 0,01$; $p_{Asn/Asn-Lys/Lys} < 0,00001$; $p_{Asn/Asn-Lys/Asn} < 0,001$). The frequency of the Lys allele in male from the main group was 73,39%, the Asn allele - 26,61% ($p_{Lys-Asn} < 0,00001$). Because of the low frequency of carriers of Asn198Asn genotype men with Lys198Asn genotypes and Asn198Asn from the main group were united

as carriers of the Asn allele. The frequency of the Asn allele in the male main group is 43,55% (n=27) ($p_{\text{Asn allele -Lys/Lys}} > 0,05$).

Comparing the frequencies of genotypes and alleles of ET-1 gene in the control group and among patients with uncomplicated EH and LVH it was found that no significant differences were defined between these groups ($p > 0,05$) (Figure 1,2).

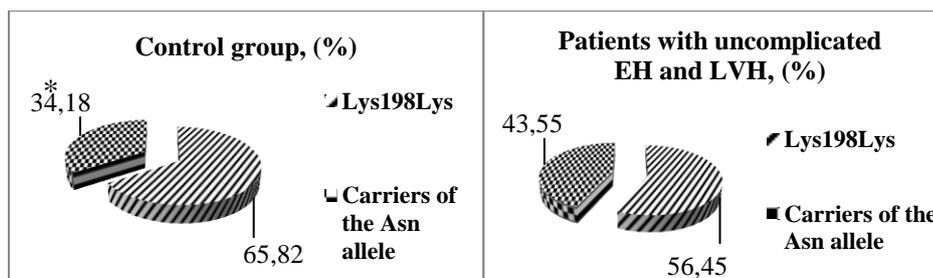


Figure 1. The distribution of ET-1 gene genotypes frequencies in men citizens of Podillia region in Ukraine in the control group and the patients with uncomplicated EH and LVH, (%)

Note: The difference is significant ($p \leq 0,05$) when compared to: * - Lys198Lys genotype within each group.

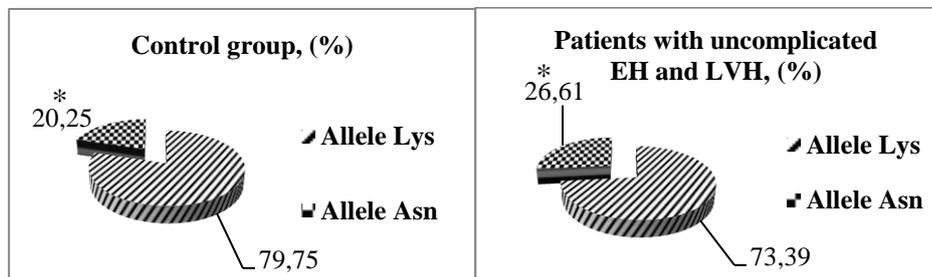


Figure 2. The distribution of the ET-1 gene alleles frequencies in men citizens of Podillia region in Ukraine in the control group and the patients with uncomplicated EH and LVH, (%)

Note: The difference is significant ($p \leq 0,05$) when compared to: * - the Lys allele within each group.

The odds ratio was calculated to assess the risk of development of uncomplicated EH with LVH in carriers of different ET-1 genotypes in men citizens of Podillia region in Ukraine aged 40-60 years old. It was established that inherited the ET-1 gene variant was not associated with the risk of development of uncomplicated EH (for genotypes the general model for imitation is not significant $\chi^2 = 2,40$; $p = 0,3$; odds ratio OR < 1; alleles multiplicative model for

imitation is not significant $\chi^2=2,73$, $p=0,1$; odds ratio OR <1) after calculating the number of statistical parameters using the calculator gene expert.

The plasma ET-1 level in the control group was $1,79\pm 0,08$ fmol/ml. In patients with uncomplicated EH and LVH average concentration of plasma ET-1 was $12,59\pm 0,22$ fmol/ml and was significantly higher than in the control group ($p<0,000001$). But it was no significant difference in ET-1 plasma concentrations in different stages of hypertension in patients with uncomplicated EH and LVH ($p>0,05$) (Table 1).

Table 1

Plasma ET-1 levels in men with different stages of uncomplicated EH with LVH (M \pm m, fmol/ml)

Stages of uncomplicated EH with LVH	ET-1 levels
1. 1 stage	$12,53\pm 0,31$
2. 2 stage	$13,36\pm 0,41$
3. 3 stage	$12,94\pm 0,40$
$p>0,05$	p_{2-1} ; p_{3-1} ; p_{3-2}

It was interesting to find the difference in concentrations of ET-1 in carriers of various genotypes of the ET-1 gene. Patients with uncomplicated EH and LVH have significantly higher concentration of ET-1 comparing with patients in the control group, carriers of all genotypes of the ET-1 gene ($p<0,0000001$). In both control group and patients with uncomplicated EH and LVH the highest concentration of ET-1 was found in carriers of the Asn allele of the ET-1 gene and the lowest level was found in homozygote carriers of Lys198Lys genotype ($p<0,0000001$) and that means that the presence of the Asn allele in the genotype of the ET-1 gene is associated with a higher plasma concentration of the peptide (Table 2).

Table 2

Plasma ET-1 levels in the control group and in the patients with uncomplicated EH and LVH with different genotypes of the ET-1 gene (M \pm m, fmol/ml)

Genotypes of the ET-1 gene	Control group	Uncomplicated EH with LVH	$p<0,0000001$
Lys198Lys	$1,41\pm 0,05$ (n=52) (1)	$11,58\pm 0,23$ (n=35) (2)	p_{2-1}
Carriers of the Asn allele	$2,53\pm 0,12$ (n=27) (3)	$13,90\pm 0,22$ (n=27) (4)	p_{4-3}
$p<0,0000001$	p_{3-1}	p_{4-2}	

There were established ET-1 levels for screening diagnosis of uncomplicated EH with LVH in men citizens of Podillia region in Ukraine that

can be applied during examining of large groups of people to identify persons who require to be conducted full examination including ultrasound of the heart and identify the presence of EH:

- The ET-1 level $\geq 7,05$ fmol/ml (sensitivity - 90%, specificity – 90,3%, correctness – 96,1%, false negative answer - 5%, false positive answer – 15,26%) can diagnose uncomplicated EH with LVH in males without taking into account the genotype of the ET-1 gene.

The results indicated that the presence of the Asn allele in the genotype of the ET-1 gene is associated with higher plasma concentrations of peptide so it was decided to calculate the ET-1 boundary levels for carriers of the different genotype of the ET-1 gene:

- The ET-1 level $\geq 6,32$ fmol/ml (sensitivity - 92%, specificity – 85,2% correctness – 87,13%, false negative answer - 7%, false positive answer – 11,92%) can diagnose uncomplicated EH with LVH in males with the homozygote Lys198Lys genotype of the ET-1 gene;

- The ET-1 level $\geq 8,12$ fmol/ml (sensitivity – 85,12%, specificity – 68,36% correctness – 87%, false negative answer – 1,45%, false positive answer – 26,84%) can diagnose uncomplicated EH with LVH in males the Asn allele carriers of the ET-1 gene.

Discussion

According to the results obtained in a Japanese study in healthy individuals of white and Japanese race, it was demonstrated that men and women (mixed group on gender) significantly more common had Lys198Lys genotype of the ET-1 gene [4]. In healthy American Negro and white races with burdened heredity by cardiovascular disease dominates the Lys allele [30]. L.O. Minushkina [2008], I.R. Petrova [2004] show that among patients of both sexes with both complicated and uncomplicated EH, residents of Yakutia, there is a high frequency of Lys198Lys genotype of the ET-1 gene carriers than among patients with EH, Moscow residents [14,20]. However, the analysis of frequency distribution of genotypes polymorphism of the ET-1 gene in men residents of Kazakhstan has shown that Lys198Lys genotype in patients with EH occurs 1.3 times less than in healthy individuals. Heterozygous Lys198Asn variant is equally common in patients with EH and in control group. Asn198Asn genotype was identified only in patients with EH [10]. The abovementioned data are slightly different from our results. It was determined that Lys198Lys genotype and the Lys allele of the ET-1 gene dominates in both men from control group and patients with uncomplicated EH and LVH residents of Podillia region in Ukraine.

The employees of the Department of Internal Medicine of the Medical Faculty №2 Vinnytsia National Pirogov Memorial Medical University defined levels of ET-1 in plasma in individuals of different sexes. It was established that the highest plasma concentration of ET-1 was registered in women postmenopausal age with EH who had had myocardial infarction and stroke in

anamnesis, and complicated with chronic heart failure with saved and reduced systolic function than with uncomplicated EH with LVH, EH without LVH and patients from control group [21,25,31]. The highest level of ET-1 in plasma was determined in men inhabitants of Podillia region in Ukraine with EH and LVH than in men with EH without LVH and patients from the control group [28].

The Department of Internal Medicine №1 Vinnytsia National Pirogov Memorial Medical University are studying ET-1 as a marker of ED in patients with EH. V.K. Serkova and N.M. Gorobec have shown that in men and women with hypertension, regardless of its causes, accompanied by a significant increase of plasma level of ET-1. Higher plasma concentration of ET-1 in patients with renal genesis of hypertension than in patients with EH can be used as differential diagnostic criteria of genesis of hypertension [26]. N.O. Kuzminova investigated that male and female patients with uncomplicated EH and LVH have higher level of vasoconstrictor ET-1 than healthy persons [17].

Similar results were obtained in the Japanese female population where high plasma levels of ET-1 was determined in people who considered themselves healthy and did not know that they had increased blood pressure [23]. It was found that in Italian men and women the plasma concentration of vasoconstrictor is significantly higher in patients with EH than in healthy individuals but is significantly lower than in patients with secondary (renal) hypertension [33]. S.V. Liamina has shown that in Russian young men (20-45 years old) with I degree hypertension, level of vasoconstrictor ET-1 in plasma is increased in 2.4 times than in healthy men and it is growing with increasing duration of hypertensive history [24]. The abovementioned results are similar to the results received in this study, where the plasma levels of ET-1 in men inhabitants of Podillia region in Ukraine with uncomplicated EH and LVH is significantly higher than in control group patients. However, the opposite data was also published. A. Hoffman [1994], B. Halawa [1999], C. Letizia [1999], L. Aziza [2011] showed no significant differences in average levels of ET-1 between patients with EH and control group [5,11,12,18]. In addition, B. Halaw showed that plasma levels of ET-1 was significantly higher in patients with severe hypertension than in patients with mild and moderate hypertension [11].

The information about plasma peptide concentrations in patients with different genotypes of the ET-1 gene is not enough. E.N. Berezykova demonstrated that Russian men and women (Tomsk city, Novosibirsk city) who are carriers of Asn198Asn genotype had elevated levels of ET-1 in plasma than carriers of Lys198Lys genotype. In carriers of Lys198Asn genotype the level of ET-1 in plasma was intermediate but there was not found significant differences between carriers of Asn198Asn genotype and Lys198Lys genotype. So carriers of the Asn allele have a higher plasma level of ET-1 while Lys198Lys genotype is associated with the lowest level of the peptide in the blood [7]. In our research work similar results were received, in both the main and in the control groups the highest concentration of ET-1 was found in carriers of the Asn allele of the ET-1 gene, the lowest peptide was identified in men with Lys198Lys genotype. At the

same time, S. Tanaka et. al. showed no difference in the levels of ET-1 at Lys198Asn polymorphism on cell cultures [29].

The literature data about plasma levels of ET-1 in patients with different severity of EH and in carriers of different genotypes of the ET-1 gene differ in some ways. Perhaps, it is the result of methods formation for population studies (persons of different sexes and different age groups were included, etc.). This requires further research on the basis of well-chosen groups of patients with EH.

Conclusions

1. Lys198Lys genotype and the Lys allele of the ET-1 gene dominate among control group and patients with uncomplicated EH and LVH, residents of Podillia region in Ukraine, aged 40-60 years old.
2. The carriers of the Asn allele of the ET-1 gene have significantly higher plasma levels of the aforementioned peptide among the control group and in the patients with uncomplicated EH and LVH.
3. There were estimated ET-1 boundary levels that can be used for screening of large contingents of people for early diagnosis of uncomplicated EH with LVH for the Asn allele carriers and homozygote carriers of Lys198Lys genotype of the ET-1 gene.

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Ethical principles. All the patients have given their written informed consent for participation in the study.

Conflict of Interest: The authors declare no conflict of interest.

Reference List

1. A.A. Abrahamovych, A.F. Faynyk, A.V. Nechay, Mechanisms of endothelial dysfunction and its role in the pathogenesis of coronary heart disease Ukrainian Journal of Cardiology 4 (2007), 81--87.
2. E.N. Amosova, Metabolic therapy of myocardial damages caused by ischemia: a new approach to the treatment of coronary artery disease and heart failure, Series "To help the practitioner", 2 (2010), 8 c.
3. M.Y. Antomonov, The calculation of boundary (critical) levels of acting accounting factors for different types of data obtained in hygienic research, Health of settlements, 43 (2004), 573-579.

4. T. Asai, T. Ohkubo, T. Katsuya et al., Endothelin-1 Gene Variant Associates With Blood Pressure in Obese Japanese Subjects, *Hypertension*, 38 (2001), 1321-1324.
5. L. Aziza, M. Sja'bani, S. Mubarika Haryana et al., The Relationship Between Endothelin-1 and Hypertension on Mlati Population, Sleman, Yogyakarta, Indonesia, *J Indon Med Assoc* 61 (2011), 237–242.
6. A.E. Barden, C.E. Herbison, L.J. Beilin et al., Association between the endothelin-1 gene Lys198Asn polymorphism blood pressure and plasma endothelin-1 levels in normal and pre-eclamptic pregnancy, *Journal of Hypertension* 19 (2001), 1775-1782.
7. E.N. Berezikova, Clinical and genetic and neurohormonal mechanisms of ischemic remodeling, myocardial apoptosis and heart failure: an innovative strategy of personalized diagnosis, prevention and treatment: Abstract of Dis. PhD, 14.01.05, Tomsk, 2014, 49.
8. G.M. Chairperson, R.F. Chairperson, K. Narkiewicz, et al, 2013 ESH/ESC Guidelines for the management of arterial hypertension, *Journal of Hypertension*, 31(7) (2013), 1281-1357.
9. M. Corizzato, R. Sega, M. Ferrario et al., Possible interaction of environment and genetic factors in work-related diseases: the case of hypertension, *Med. Lab.*, 96 (2005), 467-482.
10. A.U. Dzholdasbekova, A.E. Gaipov, The Association Between Polymorphism of Lys198Asn of the Endothelin-1 Gene and Arterial Hypertension Risk in Kazakh People, *Eur J Gen Med*, 7(2) (2010), 187-191.
11. B. Halawa, The level of plasma endothelin-1 in patients with essential hypertension, *Pol Merkur Lekarski*, 7 (38) (1999), 55-57.
12. A. Hoffman, E. Grossman, D. S. Goldstein et al., Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt sensitivity, *Kidney Int.*, 45(2) (1994), 556-560.
13. H.D. Intengan, E.L. Schiffrin, Vascular remodeling in hypertension: roles of apoptosis, inflammation and fibrosis, *Hypertension*, 38 (2001), 581–587.
14. V.I. Juncker, S.G. Grigorev, *Mathematical - statistical data processing of medical research*, St. Petersburg: MMA, (2002), 266.
15. O.V. Korkushko, V. I. Lishnevskaya, Endothelial dysfunction, *Circulation and hemostasis* 2 (2003), 4-15.
16. V.M. Kovalenko, V.M. Kornatsky, *Diseases of the circulatory system as medical and social and socio and political problem*, Kyiv, 2014, 280.
17. N.V. Kuzminova, Biochemical markers of inflammation and endothelial dysfunction in hypertensive patients, *Bulletin of Vinnytsia National University*, 3(1) (2009), 102-109.
18. C. Letizia, S. Subioli, S. Cerci et al., Effects of captopril on plasma endothelin-1 in patients with essential hypertension, *J Clin Basic Cardiol*, 2 (1999), 75–77.

19. L.O. Minushkina, Genetic factors in hypertension: the connection with the features of a current, the development of complications, treatment effectiveness: Abstract of Dis. PhD, 14.00.06, Moscow, 2008, 48.
20. R. Petrova, Clinical and genetic features of hypertensive disease in the Yakut population: Abstract of Dis. Cand. of Med. Sciences 14.00.0, Moscow, 2004, 45.
21. V.V. Petrovska, V.M. Zhebel, Parallels in structural and functional parameters of myocardium and plasma concentrations of C-natriuretic peptide and endothelin-1 in women with uncomplicated hypertension, Actual questions of pharmaceutical and medical science and practice, 3 (16) (2014), 84–87.
22. P.A. Poole-Wilson, K. Swedberg, J.G. Cleland et al., Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial, *Lancet*, 362 (2008), 7–13.
23. A. Rahman, S. Jesmin, A.K.M.A. Habib et.al., Potential association of circulatory level of endothelin-1 and hypertension in rural women in bangladesh: evidences from a community based cross-sectional study, *European Heart Journal*, 36 (2015), 24.
24. A.P. Rebrov, S.V. Lyamina, N.P. Lyamina, Diagnostically significant markers of endothelial dysfunction in young patients with hypertension, *Regional circulation and microcirculation*, 3 (2007), 59-65.
25. O.O. Sakovych, Gene polymorphism of angiotensin II type 1 and levels of natriuretic peptides in hypertensive women in postmenopausal age, uncomplicated and complicated chronic heart failure: Abstract of Dis. Cand. of Med. Sciences, 14.01.11, Kyiv, 2012, 20..
26. V.K. Serkova, N.M. Gorobec, The level of endothelin-1 in the blood in patients with essential hypertension and hypertension caused by kidney disease // *Ukrainian Therapeutic Journal*, 1 (2005), 8-10.
27. E.V. Shcheglova, Clinical and prognostic value of polymorphism of some candidate genes and markers of endothelial dysfunction in patients who underwent acute coronary syndrome: Abstract of Dis. PhD, 14.00.05, Vladikavkaz, 2008, 23.
28. S.A. Stepanets, Plasma concentrations of C-natriuretic peptide and endothelin-1 in male hypertensive patients with different genotypes of the peroxisome proliferator-activated receptor gamma, *Biomedical and biosocial anthropology* 21 (2013), 180-184.
29. C. Tanaka, K. Kamide, S. Takiuchi et al., Evaluation of the Lys198Asn and 134delA Genetic Polymorphisms of the Endothelin-1 Gene, *Hypertens Res.*, 27 (2004), 367–371.
30. F.A. Treiber, P. Barbeau, G. Harshfield et al., Endothelin-1 Gene LYS198ASN Polymorphism and Blood Pressure Reactivity, *Hypertension*, 42 (2003), 494-499.

31. G.V. Wilczynski, S. Franchuk, V.N. Zhebel, Plasma concentrations of C-natriuretic peptide and endothelin-1 in hypertensive women in postmenopausal age with different severity of disease, *Bulletin of problems of biology and medicine.*– 1(91) (2012), 100-103.
32. V.M. Zhebel, O. Sakovych, G. Wilczynskyy, O. Singh, Ukraine Patent 67486, the IPC G01N 33/48 (2006.01). Method of diagnosis of chronic heart failure in women of postmenopausal age with hypertension; the applicant and the owner the Vinnitsa National Piragov Memorial Medical University. - №u201108789; appl. 07/12/11; publ. 27.02.12, *Bulletin of №4.*
33. C. Zoccali, D. Leonardis, S. Parlongo et al., Urinary and plasma endothelin 1 in essential hypertension and in hypertension secondary to renoparenchymal disease, *Nephrol. Dial. Transplant*, 10 (8) (1995), 1320-1323.