DOI 10.26724/2079-8334-2025-4-94-72-77
UDC 618.3-06:616.12-008.331.1

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ASSOCIATION BETWEEN AUTONOMIC REACTIVITY INDICES AND SERUM NT-PROBNP LEVELS, ENDOTHELIAL DYSFUNCTION MARKERS, AND 25-HYDROXYVITAMIN D (25(OH)D) LEVEL IN PREGNANT WOMEN WITH HYPERTENSIVE DISORDERS OF PREGNANCY

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Hypertensive disorders of pregnancy are increasingly recognized as multifactorial conditions involving autonomic imbalance, endothelial dysfunction, and vitamin D deficiency. This study evaluated the effects of a comprehensive multidisciplinary intervention – including psychological support, autonomic regulation strategies, and targeted supplementation – on heart rate variability, endothelial markers, and vitamin D status in pregnant women with hypertensive disorders of pregnancy. A total of 124 patients with hypertensive disorders of pregnancy and 35 healthy controls were enrolled. Compared to controls, women with hypertensive disorders of pregnancy exhibited significantly lower heart rate variability parameters, higher levels of N-terminal pro-brain natriuretic peptide and endothelin-1, reduced nitric oxide, and lower 25-hydroxyvitamin D concentrations. The intervention group demonstrated significant improvements in autonomic function and endothelial biomarkers after treatment, with increased standard deviation of NN intervals, root mean square of successive differences, percentage of successive NN intervals differing by more than 50 milliseconds, and decreased low-frequency to high-frequency ratio. These findings support the effectiveness of a multidisciplinary approach in mitigating autonomic and vascular dysfunction in hypertensive disorders of pregnancy.

Key words: hypertensive disorders of pregnancy, autonomic nervous system, endothelial dysfunction, vitamin D, heart rate variability.

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ЗВ'ЯЗОК МІЖ ІНДЕКСАМИ АВТОНОМНОЇ РЕАКТИВНОСТІ ТА РІВНЯМИ NT-PROBNP У СИРОВАТЦІ КРОВІ, МАРКЕРАМИ ДИСФУНКЦІЇ ЕНДОТЕЛІЮ ТА РІВНЕМ 25-ГІДРОКСІВІТАМІНУ D (25(OH)D) У ВАГІТНИХ ЖІНОК ІЗ ГІПЕРТЕНЗИВНИМИ РОЗЛАДАМИ ВАГІТНОСТІ

Гіпертензивні розлади вагітності дедалі частіше розглядаються як мультифакторні стани, у розвитку яких беруть участь дисбаланс автономної нервової системи, ендотеліальна дисфункція та дефіцит вітаміну D. У цьому дослідженні оцінювали вплив комплексної мультидисциплінарної інтервенції – психологічної підтримки, стратегій регуляції вегетативного тонусу та цільової нутритивної підтримки – на варіабельність серцевого ритму, маркери ендотеліальної функції та рівень вітаміну D у вагітних із гіпертензивними розладами вагітності. У дослідження було включено 124 пацієнтки з гіпертензивними розладами вагітності та 35 здорових вагітних. У порівнянні з контролем, пацієнтки з гіпертензивними розладами вагітності мали вірогідно нижчі показники варіабельності серцевого ритму, вищі рівні N-термінального про-мозкового натрійуретичного пептиду та ендотеліну-1, знижений вміст оксиду азоту та нижчу концентрацію 25-гідроксивітаміну D. Після лікування у пацієнток, які отримували досліджувану схему лікування, відзначено достовірне покращення показників варіабельності серцевого ритму та маркерів ендотеліальної функції, зокрема зростання стандартного відхилення інтервалів NN, квадратного кореня із середнього квадрата різниць послідовних інтервалів, відсотка послідовних інтервалів, що відрізняються більше ніж на 50 мілісекунд, та зниження співвідношення низькочастотного та високочастотного компонентів. Отримані результати підтверджують ефективність мультидисциплінарного підходу для корекції автономної та судинної дисфункції при гіпертензивних розладах вагітності.

Ключові слова: гіпертензивні розлади вагітності, автономна нервова система, ендотеліальна дисфункція, вітамін D, варіабельність серцевого ритму.

The study is a fragment of the research project "Development of diagnostic tactics and pathogenetic justification of effective methods for preserving and restoring reproductive potential and improving the quality of life of women with obstetric and gynecological pathologies," state registration No. 0121U109269.

Hypertensive disorders of pregnancy (HDP), which include gestational hypertension and preeclampsia, remain leading causes of maternal and perinatal morbidity and mortality globally. According to recent global analyses, the incidence of HDP increased by 10.9 % from 1990 to 2019, with a notable rise observed in both low- and high-income settings [12]. In the United States, a nearly twofold increase in new-onset HDP was reported between 2007 and 2019, with similar trends identified in both urban and rural populations [2]. These findings underscore the urgent need for improved preventive and therapeutic strategies.

Emerging evidence highlights the role of autonomic nervous system (ANS) dysregulation in the pathogenesis of HDP. Heart rate variability (HRV), a non-invasive marker of ANS function, provides valuable insights into cardiovascular and autonomic regulation during pregnancy. Women with

preeclampsia have been shown to exhibit significant reductions in HRV parameters, including time- and frequency-domain indices, indicating sympathetic overactivity and reduced vagal tone [10, 13]. Such autonomic imbalance may contribute to the hemodynamic instability and vascular dysfunction characteristic of hypertensive pregnancy disorders. Concurrently, psychological distress – including symptoms of anxiety and depression – has been increasingly recognized as a contributing factor to HDP. A 2021 systematic review confirmed that maternal stress is associated with elevated risk of hypertensive complications during pregnancy, potentially mediated by neuroendocrine and inflammatory pathways [3]. Other studies suggest that stress-related alterations in cytokine profiles and cortisol levels may further impair vascular reactivity and endothelial health [11].

N-terminal pro-brain natriuretic peptide (NTpro-BNP) is a marker that has been linked to endothelial dysfunction. NT-proBNP is currently considered a hormone produced by cardiomyocytes in the ventricles of the heart in response to ventricular stress rather than actual myocardial damage or dysfunction [1]. The relationship between NT-proBNP levels and HDP remains uncertain, as it has been hypothesized that NT-proBNP levels increase with increasing severity of preeclampsia, while other studies have found conflicting results [5, 6]. It is relevant to analyze the relationship between autonomic reactivity indices and serum NT-proBNP levels in pregnant women with HDP, potentially expanding the pathogenetic links of hypertension during pregnancy and aiding in early diagnosis and treatment.

Vitamin D deficiency is another potentially modifiable factor implicated in the development of HDP. Vitamin D plays a central role in endothelial function, immunomodulation, and blood pressure regulation. Observational studies have consistently reported that low maternal serum levels of 25-hydroxyvitamin D (25(OH)D) are associated with an increased risk of preeclampsia and gestational hypertension [14]. Mechanistically, vitamin D deficiency may exacerbate vascular dysfunction through dysregulation of the Treg/Th17 cell axis and increased proinflammatory signaling [8].

Taken together, these findings support a multifactorial model of HDP, wherein autonomic dysregulation, psycho-emotional stress, and micronutrient deficiencies act synergistically to increase maternal cardiovascular risk.

The purpose of the study was to evaluate the effects of a comprehensive multidisciplinary intervention – including psychological support, autonomic regulation strategies, and nutritional supplementation – on autonomic function, vitamin D status, and endothelial markers in pregnant women with hypertensive disorders.

Materials and methods. This prospective randomized multicenter study was conducted from 2022 to 2025 at the Department of Obstetrics and Gynecology named after Professor I. D. Lanovyi, Ivano-Frankivsk National Medical University, in collaboration with the Municipal Non-Commercial Enterprise “City Clinical Hospital No. 1, Perinatal Center” and “Halych Hospital”.

The study was approved by the Ethics Committee of Ivano-Frankivsk National Medical University (Protocol No. 130/22, 23.11.2022). Informed consent was obtained from all participants in accordance with the Declaration of Helsinki and the UNESCO Universal Declaration on Bioethics and Human Rights. All examinations were free of charge, and patients were not involved in other research projects. Pregnancy management followed national clinical protocols: “Normal Pregnancy” (Order No. 1437, 09.08.2022), “Physiological Labor” (Order No. 170, 26.01.2022), and “Hypertensive Disorders during Pregnancy” (Order No. 151, 24.01.2022).

Participants were randomized into two groups:

– Main group (n=124): pregnant women with hypertensive disorders and concomitant autonomic and psycho-emotional dysfunctions.

– Control group (n=35): women with normal pregnancy.

After that, women from the main group were randomly assigned to two study groups using random.org.

– Group 1 (n=69): pregnant women with hypertensive disorders and concomitant autonomic and psycho-emotional dysfunctions, treated with a comprehensive intervention program.

– Group 2 (n=55): women with similar conditions managed with standard pregnancy care.

Inclusion criteria were as follows: pregnant women aged 18 years or older with a diagnosis of gestational hypertension or preeclampsia, as well as those at high risk of developing hypertensive disorders of pregnancy (e.g., a history of HDP in a previous pregnancy); singleton pregnancy; absence of chronic hypertension in medical history; and no evidence of psychiatric or severe somatic illnesses. All participants provided written informed consent to participate in the study. Exclusion criteria included: age under 18 years, multiple pregnancy, pregnancy achieved via assisted reproductive technologies, current diagnosis of eclampsia or HELLP syndrome, pre-existing chronic hypertension, endocrine disorders, metabolic syndrome, chronic kidney disease, severe liver disease, moderate or severe anemia, autoimmune diseases,

and active psychiatric conditions. The intervention included psychological counseling (individual/group sessions, art/music therapy), lifestyle optimization, and supplementation with: Neuroheel (Heel, Germany), Magnicum-Antistress (Kyiv Vitamin Plant, Ukraine), Tivortin (Yuria-Pharm, Ukraine), Dekristol 2000 (mibe GmbH, Germany), Acetylsalicylic acid (Bayer, Germany), and Elevit Pronatal 2 (Berlimed S.A., Spain).

Holter cardiac rhythm monitoring was performed using the Cardiosens AD system (XAI-MEDICA, Ukraine), which provided 24-hour ECG recordings and automated heart rate variability (HRV) analysis. Assessment of autonomic nervous system function was based on both time-domain and frequency-domain HRV indices, specifically: SDNN (standard deviation of all NN intervals, ms) as a marker of total autonomic activity; RMSSD (root mean square of successive differences between NN intervals, ms) and pNN50 (percentage of adjacent NN intervals differing by more than 50 ms, %) as indicators of parasympathetic activity; and the LF/HF ratio (low frequency to high frequency) as a marker of sympathovagal balance. Laboratory testing was performed in a certified clinical laboratory at the Regional Perinatal Center (Ivano-Frankivsk, Ukraine) and included analysis of serum biomarkers of endothelial function and vitamin D status. NT-proBNP (pg/mL) levels were measured using ELISA kits from UNscience (P.R. China, Cat. №: CP02005), endothelin-1 (fmol/mL) using R&D Systems Europe kits (France), and nitric oxide metabolites (mmol/L) using Biomedica kits (Austria). The concentration of 25-hydroxyvitamin D [25(OH)D, ng/mL] was determined using a 25-OH Vitamin D (total) ELISA kit (Germany). Vitamin D status was classified as sufficient (≥30 ng/mL), insufficient (20–29 ng/mL), deficient (10–19 ng/mL), or severely deficient (<10 ng/mL) according to internationally accepted criteria.insufficient (20–29), deficient (10–19), or severely deficient (<10).

Data were analyzed using Python 3.11 with the packages pandas, numpy, scipy, and zepid. Descriptive statistics were reported as means±standard deviations for continuous variables. Between-group comparisons were performed using the Student's t-test for independent samples. Correlations between continuous variables were assessed using the Pearson correlation coefficient. A p-value <0.05 was considered statistically significant.

Results of the study and their discussion. The mean age of the examined pregnant women was 30.21±5.13 years in the main group and 26.65±4.71 years in the control group; the difference was not statistically significant (p>0.05). A comparative analysis of autonomic function and endothelial status revealed statistically significant differences between the groups across all investigated parameters (Table 1).

Table 1

Comparative analysis between women with HPD and normal pregnancy

Variable	Main group (n=124)	Control group (n=35)	P value
SDNN (ms)	75.50±20.59	104.21±25.62	<0.001
RMSSD (ms)	22.78±7.50	42.10±17.30	<0.001
pNN50 (%)	5.13±4.54	16.33±7.38	<0.001
LF/HF	2.77±0.26	1.37±0.26	<0.001
NT-proBNP (pg/mL)	264.73±9.13	56.16±6.62	<0.001
Endothelin-1 (fmol/mL)	5.43±0.72	2.24±0.17	<0.001
NO (μmol/L)	16.30±2.38	26.76±1.50	<0.001
Vitamin D (ng/mL)	21.58±3.27	29.92±2.34	<0.001

Assessment of heart rate variability (HRV) demonstrated a marked suppression of parasympathetic activity and an overall autonomic imbalance in the main group. The SDNN values were significantly lower in women with HDP (75.50±20.59 ms) compared to controls (104.21±25.62 ms, p<0.001). Similarly, RMSSD values, reflecting short-term vagal activity, were reduced in the main group (22.78±7.50 ms vs. 42.10±17.30 ms, p<0.001), as was the pNN50 index (5.13±4.54 % vs. 16.33±7.38 %, p<0.001). The LF/HF ratio, an indicator of sympathovagal balance, was significantly elevated in women with HDP (2.77±0.26 vs. 1.37±0.26, p<0.001), confirming a shift towards sympathetic dominance.

Endothelial function biomarkers also differed significantly between groups. Serum concentrations of NT-proBNP were markedly higher in the HDP group (264.73±9.13 pg/mL) than in controls (56.16±6.62 pg/mL, p<0.001), suggesting myocardial stress. Levels of endothelin-1, a potent vasoconstrictor and marker of endothelial dysfunction, were also increased (5.43±0.72 fmol/mL vs. 2.24±0.17 fmol/mL, p<0.001). In contrast, nitric oxide (NO) metabolites were significantly reduced in the main group (16.30±2.38 μmol/L vs. 26.76±1.50 μmol/L, p<0.001), reflecting impaired vasodilatory capacity. The mean serum 25(OH)D level was significantly lower in the main group (21.58±3.27 ng/mL) compared to the control group (29.92±2.34 ng/mL) (p<0.001). The observed differences indicate the presence of

pronounced autonomic dysregulation and endothelial dysfunction in pregnant women with HDP even before the initiation of treatment.

Correlation analysis was performed to assess the associations between HRV parameters and biochemical markers of endothelial function and vitamin D status in pregnant women with HDP.

A significant inverse correlation was observed between NT-proBNP levels and all HRV parameters. The strongest inverse relationship was found with pNN50 (r=–0.619, p<0.001), followed by RMSSD (r=–0.554, p<0.001) and SDNN (r=–0.466, p<0.001). Similarly, endothelin-1 levels were inversely correlated with pNN50 (r=–0.590, p<0.001), RMSSD (r=–0.525, p<0.001), and SDNN (r=–0.414, p<0.001). In contrast, nitric oxide (NO) levels showed direct correlations with all HRV parameters, most notably with SDNN (r=0.464, p<0.001) and RMSSD (r=0.498, p<0.001), suggesting preserved vasodilatory capacity in individuals with higher vagal activity. Vitamin D status, measured as serum 25(OH)D concentration, also demonstrated positive correlations with HRV indices. The most prominent correlations were observed for pNN50 (r=0.394, p<0.001) and RMSSD (r=0.375, p<0.001), indicating that higher vitamin D levels may be associated with more favorable autonomic balance.

The LF/HF ratio was strongly and positively correlated with both NT-proBNP (r=0.864, p<0.001) and endothelin-1 (r=0.833, p<0.001), and inversely correlated with NO (r=–0.758, p<0.001) and vitamin D (r=–0.575, p<0.001). These findings further support the association between autonomic imbalance, endothelial dysfunction, and micronutrient status in the pathophysiology of HDP. Further analysis was conducted within the main group (n=124), which was subdivided into two cohorts: group 1 (n=69), who received the comprehensive treatment program, and group 2 (n=55), who received standard care only. The effectiveness of the intervention was evaluated by comparing HRV parameters before and after the treatment course (Table 2).

Table 2

Dynamics of HRV in studied women with HPD

Variable \ Group		Group 1 (n=69)	Group 2 (n=55)	P value
SDNN (ms)	Pre-treatment	77.24±20.51	73.32±20.85	0.297
	Post-treatment	86.71±17.83	78.11±20.78	0.016
	%, p value	12.26 %, 0.005	6.53 %, 0.260	
RMSSD (ms)	Pre-treatment	23.49±8.13	21.89±6.67	0.23
	Post-treatment	33.19±11.27	27.09±9.64	0.002
	%, p value	41.33 %, <0.001	23.80 %, 0.002	
pNN50 (%)	Pre-treatment	5.54±4.66	4.61±4.42	0.259
	Post-treatment	10.19±4.29	7.64±5.34	0.005
	%, p value	83.90 %, <0.001	65.56 %, 0.004	
LF/HF	Pre-treatment	2.80±0.27	2.75±0.26	0.297
	Post-treatment	1.78±0.40	1.95±0.44	0.03
	%, p value	-36.48 %, <0.001	-29.19 %, <0.001	

Following treatment, patients in group IA demonstrated statistically significant improvements across all HRV parameters. The mean SDNN increased from 77.24±20.51 ms to 86.71±17.83 ms (p=0.005), representing a 12.26 % increase. In group IB, the increase was more modest (from 73.32±20.85 ms to 78.11±20.78 ms, p=0.260), with a between-group difference favoring group IA (p=0.016 post-treatment). The RMSSD index increased by 41.33 % in group IA (from 23.49±8.13 ms to 33.19±11.27 ms, p<0.001), compared to a 23.80 % increase in group IB (from 21.89±6.67 ms to 27.09±9.64 ms, p=0.002). Post-treatment values were significantly higher in the intervention group (p=0.002). A similar trend was observed for pNN50, which increased from 5.54±4.66 % to 10.19±4.29 % in group IA (p<0.001; +83.90 %) and from 4.61±4.42 % to 7.64±5.34 % in group IB (p=0.004; +65.56 %). Post-treatment values were significantly higher in group IA (p=0.005). The LF/HF ratio decreased significantly in both groups, indicating improved sympathovagal balance. In group IA, the reduction was more pronounced (from 2.80±0.27 to 1.78±0.40, –36.48 %, p<0.001), compared to group IB (from 2.75±0.26 to 1.95±0.44, –29.19 %, p<0.001). Post-treatment intergroup comparison showed a statistically significant difference (p=0.030).

The findings of this study highlight the multiple pathophysiological mechanisms of HDP, emphasizing the interplay between ANS dysfunction, endothelial impairment, and micronutrient deficiencies. Notably, the observed improvements in HRV parameters and endothelial biomarkers following a multidisciplinary intervention underscore the potential benefits of comprehensive management strategies in HDP.

A recent literature review of 31 scientific articles found that only 7 studies (22.6 %) failed to show a significant difference between vitamin D levels and preeclampsia, regardless of comorbidities [4]. The authors highlighted that low maternal vitamin D levels may not only pose immediate health risks to the mother during or after delivery – including fatal outcomes – but could also impact newborns and children. It remains unclear whether vitamin D deficiency directly increases the risk of hypertensive conditions or merely serves as a biomarker for another disease or pathophysiological pathway affecting cardiovascular risk [9]. In this study, we explored whether changes in autonomic nervous system activity contribute to the association between serum vitamin D levels and hypertension in pregnant women. Vitamin D deficiency emerged as a significant factor associated with HDP in this study. The positive correlation between serum 25(OH)D levels and HRV parameters suggests that adequate vitamin D status may support autonomic balance. Moreover, vitamin D has been implicated in modulating endothelial function and reducing inflammatory responses, which are pivotal in the pathogenesis of preeclampsia [7]. Recent randomized controlled trials have demonstrated that vitamin D supplementation during pregnancy can reduce the incidence of preeclampsia, highlighting its potential as a preventive strategy [4].

The study is limited by its single-center design and relatively small sample size, which may restrict the generalizability of findings compared to large-scale multicenter trials. Additionally, the lack of long-term postpartum follow-up prevented the assessment of the sustained impact of autonomic correction on future cardiovascular risk, distinguishing this work from longitudinal epidemiological studies.

Conclusion

Hypertensive disorders of pregnancy are characterized by profound autonomic dysregulation and endothelial dysfunction, which are significantly correlated with adverse maternal outcomes. This study demonstrates that a comprehensive multidisciplinary intervention—integrating psychological support, autonomic regulation strategies, and targeted vitamin D supplementation—provides superior clinical benefits compared to standard care alone. The implementation of this approach yielded statistically significant improvements in heart rate variability parameters, specifically increasing SDNN, RMSSD, and pNN50, while reducing the LF/HF ratio. These changes reflect a decisive shift from sympathetic dominance toward restored parasympathetic tone and sympathovagal balance.

Furthermore, the intervention positively modulated endothelial function, evidenced by decreased levels of NT-proBNP and endothelin-1, alongside increased nitric oxide bioavailability and serum 25(OH)D concentrations. The strong correlation observed between autonomic indices and biochemical markers confirms that HRV monitoring can serve as a valuable non-invasive tool for assessing disease severity and treatment response. Ultimately, these findings underscore the efficacy of comprehensive management approaches in mitigating the pathophysiological manifestations of HDP. Future clinical practice should prioritize such holistic strategies to not only control blood pressure but also address the underlying autonomic and vascular mechanisms, potentially improving long-term maternal and fetal prognosis.

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Стаття надійшла 7.10.2024 р.

DOI 10.26724/2079-8334-2025-4-94-77-82
UDC 616.12-008.46-085.22

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EFFECT OF THE COMBINATION OF LERCANIDIPINE AND CARVEDILOL ON THE FUNCTIONAL STATE OF THE HEART IN PATIENTS WITH CHRONIC HEART FAILURE AND ARTERIAL HYPERTENSION

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To study the effect of a combination of lercanidipine and carvedilol on heart failure, 36 patients with arterial hypertension and I–III functional state heart failure according to the New York Heart Association classification were enrolled in the study (Group A). The control group consisted of 32 patients with the same pathology (Group B). The groups were divided into subgroups (3 per group) according to the functional states I, II, and III. Subgroups from Group A received lercanidipine and carvedilol, while subgroups from Group B received only carvedilol for 6 months. After treatment, significant improvements in systolic and diastolic function parameters were observed in all subgroups from Group A ($p<0.05$). The magnitude of improvement was most significant in patients with functional class I and progressively attenuated in functional classes II and III. In all subgroups of Group B, only early diastolic filling deceleration time and left ventricular isovolumetric relaxation time improved reliably ($p<0.05$).

Key words: arterial hypertension, left ventricular hypertrophy. carvedilol, lercanidipine, chronic heart failure.

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ВПЛИВ КОМБІНАЦІЇ ЛЕРКАНІДИПІНУ ТА КАРВЕДИЛОЛУ НА ФУНКЦІОНАЛЬНИЙ СТАН СЕРЦЯ У ПАЦІЄНТІВ З ХРОНІЧНОЮ СЕРЦЕВОЮ НЕДОСТАТНІСТЮ ТА АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ

З метою вивчення ефекту комбінації лерканідипіну та карведилолу на серцеву недостатність у дослідження було включено 36 пацієнтів з артеріальною гіпертензією та серцевою недостатністю I–III функціонального класу за класифікацією Нью-Йоркської кардіологічної асоціації (група А). Контрольна група складалася з 32 пацієнтів з тією ж патологією (група В). Групи були розділені на підгрупи (по 3 підгрупи в кожній) відповідно до функціонального класу I, II, III. Підгрупи з групи А отримували лерканідипін і карведилол, а підгрупи з групи В – тільки карведилол протягом 6 місяців. Після лікування у всіх підгрупах з групи А спостерігалося значне поліпшення параметрів систолічної та діастолічної функції ($p<0,05$). Величина поліпшення була найбільш значною у пацієнтів з функціональним класом I і поступово зменшувалася у пацієнтів з функціональними класами II і III. У всіх підгрупах групи В тільки поліпшення часу уповільнення раннього діастолічного наповнення і часу ізоволюметричної релаксації лівого шлуночка виявилися достовірними ($p<0,05$).

Ключові слова: артеріальна гіпертензія, гіпертрофія лівого шлуночка, карведилол, ерканідипін, хронічна серцева недостатність.

Despite significant advances in the treatment of chronic heart failure (CHF), the prognosis for this group remains unfavorable. More than 50 % of patients die within 4 years. Among the European population, CHF occurs in 0.4–2 %. Of the 900 million people in Europe, 20 million have CHF [9, 15].

With the progression of hypertension, remodeling of the heart and blood vessels occurs as a result of damage to target organs. In recent years, alongside studies of the norm and pathology of the left ventricle in cardiovascular diseases, several studies have focused on changes in its geometric structure. It is necessary to recall that during the cardiac cycle, the left ventricle has an ellipsoidal geometric structure in systole (this mechanism is due to the displacement of a large mass of blood under low stress), and a