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Assessment of the expression of endothelial dysfunction in chronic heart failure in female patients

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ABSTRACT

Systemic endothelial dysfunction plays a key role in the pathogenesis of many cardiovascular diseases today. The article aims to assess the age-related features of the severity of systemic endothelial dysfunction in female patients with chronic heart failure. In chronic heart failure, there is an increase in the basal release of nitric oxide, which indicates that the stimulated and basal production of nitric oxide in this pathological condition is dissociated. The study included 139 patients with chronic heart failure developed due to coronary heart disease and arterial hypertension and they were divided into three age groups. The mean age was 67.1±10.6. Patients with CHF that developed as a result of various forms of cardiomyopathy and chronic rheumatic heart disease were excluded from the study. All patients underwent a comprehensive examination in the study process, which included clinical (the scale of assessment of the clinical condition) modified by [1] and the 6-minute walk test (TSW) and instrumental methods of research (EchOX). Assessment of the level of nitric oxide (NO) in patients' blood in 3 age groups showed a significant increase in its level in all groups compared to the control indicators in all groups. At the same time, the highest values were observed in the group of elderly patients. The lowest average values were determined in the middle-aged group. According to the results of the study, it can be concluded that improving the availability of endothelial nitric oxide by stimulating endothelial NO synthase is one of the objectives of effective treatment of chronic heart failure in female patients.

1. INTRODUCTION

Recent studies show that several endogenous mechanisms play a role in developing and progressing chronic heart failure (CHF). One of the leading mechanisms of neurohumoral systems regulating the activity of the cardiovascular system is the renin-angiotensin, sympathoadrenal and endothelin systems [1- 4]. Endothelial dysfunction (ED) is understood as a decrease in vascular dilatation and an increase in vasoconstriction, accompanied by the activation of the cytokine system and a violation of the thrombosis resistance of the vascular wall. Its essence can be formulated as follows: those substances under normal conditions are vasodilators in ED can no longer have their relaxing effect and sometimes can even cause the opposite

effect in the form of a spasm [5- 7]. Nitric oxide (NO) is a powerful endogenous vasodilator, causing relaxation of the smooth muscles of the vascular walls, participating in systemic and pulmonary vascular resistance, blood coagulation processes, and reducing platelet aggregation and their adhesion [8], [9]. A normally functioning endothelium is characterized by continuous basal NO production by endothelial NO synthase (eNOS). NO-synthase exists in the form of 3 main isoforms named after the type of cells in which they were found. According to the physiological properties, NO synthases are divided into constitutive, i.e., enzymes with stable activity- neuronal NO synthase (nNOS or NOS I), endothelial NO synthase (eNOS or NOS III) and inducible NO synthase (iNOS or NOS II), the activity of which is regulated by cytokines [10], [11]. When iNOS is activated, there is a prolonged increase in the NO level [12]. It is necessary to maintain a normal basal vascular tone. Treatment of CHF is one of the most urgent and complex tasks of modern cardiology. In this regard, an understanding of the importance of endothelial function in CHF and the search for new, modern methods of correcting endothelial dysfunction would allow us to achieve favourable results in treating patients with CHF and improving the quality of life and prognosis in this category of patients.

The purpose of the study. To study the age-related features of the severity of Ehlers–Danlos syndromes (EDS) in female patients with CHF FC II and III.

2. Materials and methods

The study included 139 patients with CHF that developed due to coronary heart disease (CHD) and arterial hypertension (AH). The average age was 67.1±10.6 years. Depending on the age characteristic, the patients were divided into three groups:

- Elderly group: included 69 older women (60-74 years), 35 of whom suffered from CHF FC II and 34 with CHF FC III.
- Senile age group: included 38 women of senile age (75-90 years), 19 of whom suffered from CHF FC II and 19 with CHF FC III.

The middle-aged group: included 32 middle-aged women (45-59 years), 20 of whom suffered from CHF FC II and 12 with CHF FC III.

Patients with CHF that developed as a result of various forms of cardiomyopathy and chronic rheumatic heart disease were excluded from the study.

All patients underwent a comprehensive examination in the study process, which included clinical (the scale of assessment of the clinical condition (SACC) modified by [1] and the 6-minute walk test (TSW) and instrumental methods of research (EchOX).

Electrocardiography (ECG) was performed in 12 leads on the device "SHELLERCardiovit AT 1" according to the standard method. For echocardiography (EchoCG), the SSH-160A "Toshiba" device (Japan) was used.

The work on the determination of EDS markers in the form of nitric oxide metabolites-nitrites and nitrates

- (NO) endothelial NO synthase (eNOS), inducible NO synthase (iNOS) and peroxynitrite (ONOO-)) was carried out based on the Central Research Laboratory of Tashkent Medical Academy (TMA) under the supervision of the head of the department, Candidate of Medical Sciences Komarin A. S.

The NOS activity was determined in a reaction system containing 2.5 ml of 0.1 M tris HCl buffer (pH 7.4), CaCl2 (10 mM), 10 mm L-arginine solution (NOS substrate), and 0.1 mM Nicotinamide adenine dinucleotide phosphate (NADPH2) aqueous solution. The reaction was initiated by adding 0.1 ml of blood serum. Incubation was carried out at T 37°C for 60 minutes. The reaction was stopped by adding 0.02 ml of 0.02% sodium azide (NaN) solution. When the extinction index reached 340 nm, the spectrophotometer SF-46 (Russia) was recorded. In this case, the activity of NOS was determined by the formula:

$$A = \frac{E}{k \cdot V \cdot t} \text{ мкмоль/л}$$

where, A is the activity of the enzyme, k is the calibration coefficient for NADPH2 equal to $6.52 \cdot 10^3$ mM^{-1} cm^{-1}, ΔE is the index (SF-46), V is the volume of the material (0.1 ml), t is the duration of incubation. The determination of the level of NO, its stable metabolites and the sum of nitrites and nitrates was carried out according to the method of P. P. Golikova et al. For this, 0.05 ml of 5% NH_4Cl solution and 1.5 ml of Gris reagent (solutions of 1% sulfonamide, 0.1% naphthylenediamine, 2.5% phosphoric acid (Sigma, USA)) were added to 0.1 ml of blood serum and kept at room temperature for 10 minutes. The absorption index was determined when the wavelength on the SF-46 reached 546 nm. Sodium nitrite ($NaNO^3$) was used as the standard. The calculation was carried out according to the following formula:

$$A = k*E \text{ (mmol/l)},$$

where, k is the calculation coefficient equal to 40; E is the material extinction index (nmol).

The volume of peroxynitrite was determined by oxidation (NH_2O-) after the reaction: ONOO- + NHO- - NO_2 - + NO + H_2O. For this, 0.2 ml of 1.5% aqueous hydroxylamine solution was added to 0.1 ml of blood serum. After 10 minutes, the reaction was stopped by adding 1.0 ml of a 4% ammonium molybdate solution. The following formula calculated the activity of the enzyme in micromol/l:

$$A = (E_{xol} - E_{on})*V*k \text{ (mmol/l)},$$

where, A – the volume of peroxynitrite (nmol/l), E_{xol} and E_{on} – the extinction of the studied materials, V – the volume of the material (0.1 ml of blood serum), k – calibration coefficient equal to 40.0 mM^{-1} * cm^{-1} [9].

The data obtained in the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package, including the use of built-in statistical processing functions.

3. Results

Assessment of the level of nitric oxide (NO) in patients' blood in 3 age groups showed a significant increase in its level in all groups compared to the control indicators in all groups. At the same time, the highest values were observed in the group of elderly patients. The lowest average values were determined in the middle-aged group.

The level assessment of endothelial NO synthase (eNOS) in all the studied age groups was significantly lower than the standard values. Simultaneously, patients with CHF had a decrease in its level by more than two or more times. The most pronounced decrease was observed in the group of elderly patients. The indicators of elderly patients were significantly lower than in the elderly group (Table 1).

The level of inducible NO-synthase was significantly increased compared to normal values. At the same time, its level increased by 3-4 times. The most significant increase in its level was observed in elderly patients. The indicators of elderly patients were significantly higher than in the elderly group.

Finally, the assessment of the peroxynitrite level showed that patients in all groups with CHF had a significant increase in the level toxic to cells (above 0.2).

Table 1. Comparative assessment of nitric oxide system parameters in the studied patients in different age groups

Indicators	Middle age n=32	Elderly age n=69	Senile age n=38	Control
NO (mmol/l)	16,81±1,61*	21,43±7,25*	23,09±4,73*	11,3±0,2
eNOS (mmol/l)	7,89±0,71*	6,35±2,06*	5,46±1,47*	11,6±0,4
iNOS (mmol/l)	1,81±0,22*	2,32±1,01*	2,66±0,69*	0,7±0,03
$ONOO^-$ (mmol/l)	0,35±0,07*	0,43±0,15*	0,54±0,06*	0,2±0,02

Note: * - the differences compared to the control are statistically significant.

The results of the study of the level of nitric oxide and its main metabolites showed that, in general, there is a significant increase in the level of NO metabolites in CHF. Simultaneously, the most significant growth is determined in old age and in the presence of concomitant pathology in the form of Diabetes mellitus (DM), which affects the severity of EDS. There is not so much a change in the level of nitric oxide in the blood as a dissociation of the secretion of its physiological forms in EDS. By analyzing the content of the enzymes responsible for its synthesis in the blood, it was shown that in CHF, there is a decrease in endothelial NO synthase (eNOS), which is responsible for the production of the endothelial fraction of nitric oxide, which acts selectively on the vessels. Simultaneously, the level of inducible iNOS synthase, which promotes nitric oxide production operating generically, was significantly increased in all groups. The change in the ratio in the production of various NO fractions explains the dissociation mechanism of the physiological action of the nitric oxide system in the body in CHF. In the blood of patients with CHF in all age groups, there was an increase in peroxynitrite ONOO-, which, when the permissible values for the body increase (0.2 mmol/l), has a toxic effect. In the comparative aspect, the most severe violations of the studied indicators were found in the group of elderly patients, which is explained by both systemic ED associated with the development of involutional changes and systemic ED related to CHF development.

The calculation of the correlation relationship shows that there was an average or noticeable correlation among the age of the patients. Simultaneously, it is noteworthy that the TSW and Ejection Fraction (EF) index did not show such a strong correlation with the level of the studied substances. Nevertheless, despite the average indicators in most of the patients, the correlation was pronounced, especially in elderly patients and patients with DM. Thus, it is possible to say that the level of NO, eNOS and ONOO - is an indicator of the severity of CHF.

4. Discussion

In CHF, the endothelium cannot produce NO to meet metabolic needs. As a result, the intensity of endothelium-dependent vasodilation changes, and the stimulated release of NO in response to the action of acetylcholine and bradykinin decreases [13]. It was shown that in CHF, there is an increase in basal NO release, which supports adequate tissue perfusion at rest [14], [15]. Consequently, stimulated and basal NO production is partially dissociated in patients with CHF. Altered vasodilation in CHF is more selective than generalized in moderate clinical symptoms. The impaired vasodilating effect becomes generalized with CHF progression, possibly due to defects in the guanylate cyclase system, which determines the response of

smooth muscle cells to cyclic guanosine monophosphate (cGMP) [16], [17]. The total concentration of NO-synthase may increase in CHF due to an increase in iNOS. Thus, Ageev F. T. [1] showed in their studies that as a result of EDS in CHF, ENOS suppression and a decrease in NO synthesis develop, when, against the background of a chronic decrease in blood flow in the tissues, a perversion of the vascular response to "shear stress" occurs. This process is accompanied by an increase in the concentration of free radicals that inactivate NO and an increase in the level of cyclooxygenase- dependent endothelial constriction factors that "compensate" for the dilating effect of NO. The results of foreign studies showed that in the elderly and senile age, there is a proportional decrease in NO production, which has a significant effect on the course of CHF [14- 16]. It was proved that starting from 40; there is a significant annual decrease in the blood flow rate in the brachial artery by an average of 0.21%, which is an indicator of SED [11]. It was also shown that EDS is more pronounced in postmenopausal women compared to men of this age group [17].

High levels of nitrite and nitrates in severe CHF were also detected by other researchers [18]. An increase in the final NO metabolites was determined simultaneously with eNOS deficiency and reduced endothelium-dependent vascular relaxation. The most likely explanation for the increased level of nitrite and nitrates in CHF is the increased expression of iNOS, which is not synthesized in healthy individuals. Still, cytokine-mediated production of iNOS in the myocardium and systemic circulation was proven in CHF [15]. Excessive NO formation in CHF has a compensatory value aimed at maintaining tissue perfusion. At the same time, excess NO in CHF contributes to increased endothelial dysfunction by activating apoptosis, suppressing endothelial NO, and inhibiting myocardial contractile function [16], [17]. Our study showed that assessing the level of nitric oxide and its main metabolites in female patients with CHF in 3 different age groups revealed a significant increase in nitric oxide and its main toxic metabolite, peroxynitrite ONOO. It was also proved that the manifestation of ED in CHF is the dissociation of various types of nitric oxide synthesis, which confirmed the change in the level of the main enzymes eNOS and iNOS.

5. Conclusion

The results obtained allow us to conclude that changes in the levels of NO, eNOS, iNOS, and ONOO- in chronic heart failure serve as confirmation of the development of pronounced systemic endothelial dysfunction, which is one of the links of pathogenesis that must be affected during therapy. The results suggest that one of the objectives of effective treatment of chronic heart failure in female patients is to improve the availability of endothelial nitric oxide by stimulating endothelial NO synthase.

6. Conflict of interests and contribution of authors

The authors declare the absence of apparent and potential conflicts of interest related to this article's publication and report on each author's contribution.

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8. References

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