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BIOCHEMICAL MARKERS OF ENDOTHELIAL DYSFUNCTION IN INDIVIDUALS WITH BURDEN HYPERTENSION HEREDITY AND PATIENTS WITH STAGE II HYPERTENSION

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The article presents the results of a study of the levels of biochemical markers of endothelial dysfunction in healthy individuals with a burdened heredity for arterial hypertension and patients with stage II hypertension. The state of vascular endothelium was studied by the levels of NO-synthesizing system (total, inducible and endothelial NO-synthase), the content of stable NO metabolites – S-nitrosothiols, the level of vasoconstrictor endothelin-1 and the activity of von Willebrand factor. It was noted that endothelial dysfunction develops in the serum of healthy individuals with a burdened heredity for hypertension, as evidenced by an increase in the vasoconstrictor and thrombogenic potential of the vascular endothelium, which is also observed in patients with stage II hypertension. Thus, in both groups of subjects, a significant increase in the activity of all types of NO-synthases (total, inducible and endothelial), von Willebrand factor activity and endothelin-1 levels, as well as a decrease in S-nitrosothiol levels ($p<0.001$) was found relative to healthy individuals without burdened heredity. At the same time, most of the studied parameters did not differ significantly between the groups of patients with hereditary hypertension and patients with diagnosed stage II hypertension, except for endothelin-1 levels ($p=0.011$) and a tendency to reliability in S-nitrosothiol levels ($p=0.07$). Thus, our data indicate that people with hereditary hypertension have disorders of the functional state of the vascular endothelium even before the onset and development of hypertension, which gives reason to believe that they are genetically determined.

Key words: hypertension, genetic predisposition, heredity for hypertension, disorders of endothelial functions, endothelial dysfunction, NO-synthase, S-nitrosothiols, von Willebrand factor, endothelin-1.

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БІОХІМІЧНІ МАРКЕРИ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ В ОСІБ ЗІ СПАДКОВІСТЮ ПО АРТЕРІАЛЬНІЙ ГІПЕРТЕНЗІЇ ТА ХВОРИХ НА ГІПЕРТОНІЧНУ ХВОРОБУ ІІ СТАДІЇ

В статті відображені результати дослідження рівнів біохімічних маркерів ендотеліальної дисфункції у здорових осіб з обтяженою спадковістю по артеріальній гіпертензії та пацієнтів з гіпертонічною хворобою ІІ стадії. Стан ендотелію судин вивчали за рівнями NO-синтезуючої системи (загальна, індуцибельна та ендотеліальна NO-синтази), вмістом стабільних метаболітів NO – S-нітрозотіолів, рівнем вазоконстриктора ендотеліну-1 та активністю фактора Віллебранда. Відмічено, що в сироватці крові здорових осіб з обтяженою спадковістю по артеріальній гіпертензії розвивається ендотеліальна дисфункція, свідчення чого є зростання вазоконстрикторного й тромбогенного потенціалу судинного ендотелію, що спостерігається й у хворих на гіпертонічну хворобу ІІ стадії. Так, в обох групах обстежених встановлено достовірне відносно здорових осіб без обтяженої спадковості зростання активності всіх типів NO-синтаз (загальної, індуцибельної та ендотеліальної), активності фактора Віллебранда і рівнів ендотеліну-1 та зниження рівнів S-нітрозотіолу ($p<0,001$). При цьому більшість показників, що вивчалися, достовірно не відрізнялися між групами осіб зі спадковістю по артеріальній гіпертензії та хворими з діагностованою гіпертонічною хворобою ІІ стадії, за винятком рівнів ендотеліну-1 ($p=0.011$) та тенденції до достовірності у рівнях S-нітрозотіолу ($p=0.07$). Таким чином, отримані нами дані свідчать, що у людей зі спадковістю по артеріальній гіпертензії ще до виникнення і становлення гіпертензії, виникають порушення функціонального стану судинного ендотелію, що дає підстави думати про їх генетичну детермінованість.

Ключові слова: гіпертонічна хвороба, генетична схильність, спадковість по артеріальній гіпертензії, порушення функцій ендотелію, ендотеліальна дисфункція, NO-синтаза, S-нітрозотіоли, фактор Віллебранда, ендотелін-1.

The study is a fragment of scientific research projects "Metabolic risk factors, cardiovascular remodeling and functional state of kidneys in patients with cardiovascular pathology. Possibilities of pharmacological correction", state registration No. 0119U101849 and "Cardiovascular remodeling, structural and functional state of the liver and kidneys and their relationship with cardiometabolic risk factors in patients with cardiac pathology and comorbidities. Possibilities of treatment optimization", state registration No. 0124U002036.

Despite the significant scientific advances in modern medicine, cardiovascular disease (CVD) is still the leading cause of death in the world, including in Ukraine [2, 9, 15]. Thus, according to the WHO, they cause more than a third of all deaths, and in countries with low economic development, this share is growing significantly [9, 14].

Arterial hypertension (AH) is not just a persistent increase in blood pressure (BP), but the most common cause of the "launch" of the cardiovascular continuum with the development of complications: myocardial infarction, cerebral stroke, chronic heart failure, cardiac death, which makes this pathology one

of the most pressing health problems in the world [10, 14]. The results of the STEPS study (STEPwise approach to noncommunicable disease risk factor surveillance) showed that one third of the population of Ukraine (34.8 % of respondents) had hypertension or took antihypertensive drugs, with the prevalence of hypertension increasing sharply with age [5].

The functional state of the vascular endothelium is an important factor in cardiovascular health, as the endothelium is an important endocrine organ that regulates vascular tone, smooth muscle cell proliferation, blood flow, angiogenesis, immune response and hemostasis, ensuring the integrity and stability of the vascular wall. According to the literature, on the one hand, endothelial dysfunction is a consequence of the negative impact of cardiovascular risk factors, including hypertension, on the vascular endothelium, and on the other hand, it causes an increase in blood pressure due to an imbalance in the ratio of vasodilators (primarily nitric oxide) and vasoconstrictors in favor of the latter, proliferation of smooth muscle cells, and increased vascular wall stiffness [8, 13]. Therefore, an attempt to resolve the issue of what is the primary endothelial dysfunction or the onset of hypertension is relevant.

The purpose of the study was to evaluate the functional state of the vascular endothelium in healthy individuals with burden hypertension heredity and patients with stage II hypertension.

Materials and methods. The patients included in the study sought consultations with cardiologists at Vinnytsia Clinical City Hospitals #1 and #2, Vinnytsia Regional Clinical Hospital named after M.I. Pirogov, and Central Clinical Hospital #5 of the Southern Ukrainian Railways in Kharkiv

The study included 231 patients with stage II hypertension (105 men and 126 women), whose average age was 52.3±1.2 years, with an average duration of hypertension of 9.7±0.45 years. In 121 of 231 patients, concomitant stable coronary heart disease (CHD) was diagnosed – functional class II-III angina pectoris (FC), the average duration of which was 4.21±1.6 years. Moreover, 30 healthy subjects of equivalent age and gender lacking any familial history of hypertension (control group) and 23 healthy individuals of similar age and gender, free from hypertension and coronary heart disease, yet with a familial history of hypertension (comparison group) – which included those with direct relatives (first degree) possessing a validated record of myocardial infarction due to hypertension or cerebral stroke – were evaluated.

Non-inclusion criteria were: presence of AH stages I and III, severe cardiac rhythm and conduction disorders, kidney or liver disease with impaired function, diabetes mellitus, obesity of the third degree, heart defects, severe chronic heart failure (II-B-III stages according to the classification of M.D. Strazhesko and V.H. Vasilenko), chronic respiratory diseases with respiratory failure. After the examination, patients with established symptomatic hypertension were excluded.

Blood sampling from the cubital vein for clinical and biochemical examination was performed on the first day of admission to the hospital. At the initiation of the study, in conjunction with comprehensive clinical evaluations, all participants underwent an array of assessments including blood glucose levels, electrolyte concentrations (K^+, Na^+), and measurements of urea and creatinine. Additionally, they were subjected to an evaluation of the prothrombin index or INR, total protein quantification, fibrinogen analysis, and an examination of total bilirubin along with its distinct fractions. Furthermore, the activities of alanine and aspartate aminotransferases were meticulously assessed.

The functional state of the vascular endothelium was studied by the state of the NO-synthesizing system (total NO-synthese, inducible NO-synthese, and endothelial NO-synthese), the content of stable NO metabolites - S-nitrosothiols, the level of vasoconstrictor endothelin-1, and the activity of von Willebrand factor.

The content of stable nitric oxide metabolites (S-nitrosothiols (RS-NO)) and NO synthase activity (total NO synthase (NOS total) and inducible NO synthase (i-NOS)) were evaluated in blood plasma using the spectrophotometric method. The activity of endothelial NO synthase (e-NOS) was calculated by the formula: e-NOS = NOS total – i-NOS [3]. The activity of von Willebrand factor (vWF) was determined in plasma by the method using ristomycin on a photoelectrocolorimeter.

The level of endothelin-1 (ET-1) in the blood of patients was studied by enzyme-linked immunosorbent assay (ELISA) using a set of special reagents (Endotelin-1 kit, manufactured by DRG (USA)).

Statistical calculations were performed using Microsoft Excel and Statistica for Windows 10.0.

Results of the study and their discussion. The analysis of indicators of the functional state of the vascular endothelium in patients with a burdened heredity for hypertension and patients with “isolated” stage II hypertension revealed the presence of endothelial dysfunction in both groups, which was characterized by a significant increase in the enzymatic activity of total NO-synthase, mainly due to

inducible, endothelin-1 concentration and von Willebrand factor activity, and a decrease in the level of physiologically active NO depots (S-nitrosothiols) ($p<0.01$). It should be noted that in addition to higher levels of endothelin-1 ($p=0.016$) and slightly lower levels of S-nitrosothiol ($p=0.07$) in patients with stage II hypertension without CHD, there was no significant difference in these parameters between the groups of patients with existing hypertension and those with a genetic predisposition to it but normal blood pressure ($p>0.05$) (Table 1).

Table 1

The levels of biochemical markers of endothelial dysfunction in the studied groups

Parameter	Control group (n=30)	Comparison group (n=23)	Patients with stage II AH without CHD (n=110)	P
NOS total pmol/min.*mg protein	1.12 (0.99; 1.38)	2.00* (1.77; 2.21)	2.00* (1.84; 2.22)	ns
e-NOS, pmol/min.*mg protein	0.77 (0.68; 0.87)	0.86* (0.79; 1.00)	0.88* (0.72; 1.06)	ns
i-NOS, pmol/min.*mg protein	0.32 (0.24; 0.53)	1.11* (1,02; 1,21)	1.11* (0.98; 1.21)	ns
RS-NO, mmol/l	0.57 (0.49; 0.64)	0.47* (0.39; 0.49)	0.39* (0.21; 0.53)	0.07
ET-1, pg/ml	4.01 (3.42; 4.69)	9.15* (7.15; 11.92)	13.18* (9.74; 21.49)	0.016
vWF, %.	92.4 (72.1; 104.2)	126.9* (100.5; 143.4)	132.4* (97.4; 147.2)	ns

Notes: * – the probability of differences in indicators in relation to the control group ($p<0.01$); P – the probability of differences between the comparison group and patients with AH; ns – no significant difference ($p>0.05$).

As is evident from table 1, i-NOS activity was more than three times higher than in the control group in both patients with stage II hypertension without CHD and normotensive subjects with a hereditary history of hypertension without a significant difference between these groups ($p>0.05$). The activity of e-NOS in these groups of subjects was also significantly higher than in the control group ($p<0.01$), but the degree of its increase was much less. There was no significant difference in e-NOS activity between the comparison group and patients with stage II AH ($p>0.05$).

S-nitrosothiols are physiologically active NO depots and can serve as an additional non-enzymatic source of free NO in conditions of its increased demand, for example, in high blood pressure. Thus, the mean levels of S-nitrosothiol (RS-NO) in the serum of patients with AH without CHD were significantly lower than those in healthy donors ($p<0.0001$). When assessing the levels of S-nitrosothiols in practically healthy individuals without hypertension and CHD, but with a heredity for hypertension, a similar significant decrease was noted (by 17.5%, at $p=0.002$) relative to the values in healthy people who did not have a heredity for hypertension. When comparing the levels of RS-NO in patients with stage II hypertension without CHD and healthy individuals with a burdened heredity for hypertension, only a tendency to reliability was noted ($p=0.07$).

The level of ET-1 in the blood serum of individuals with heredity for hypertension significantly increased in relation to the indicators in healthy people: 9.15 (7.15; 11.92) pg/ml vs. 4.01 (3.42; 4.69) pg/ml, respectively ($p<0.0001$), although it did not reach the values in patients with stage II hypertension without CHD – 13.18 (9.74; 21.49) pg/ml, ($p=0.016$). It is possible that the production of vasopressor substances in patients with hereditary hypertension begins long before the clinical manifestations of hypertension (increased blood pressure) and causes activation of both NO-producing systems and increased release of NO from active physiological depots, which in this case are represented by S-nitrosothiols.

The activity of von Willebrand factor significantly increased in patients with AH without CHD and in patients with hereditary hypertension compared with controls (see table 1), but without a significant difference between the groups ($p>0.05$), indicating an increase in the thrombogenic potential of the vascular wall. Significant correlations were found between the activity of VWF, on the one hand, and the level of ET-1, NOtotal and eNOS activity, on the other ($r=0.69$, $p<0.0001$, $r=-0.45$, $p<0.0001$ and $r=-0.48$, at $p<0.0001$, respectively), which generally indicated a compromise of various endothelial functions known as endothelial dysfunction.

Since hypertension is not just a pathology characterized by an increase in blood pressure, but also one of the main factors of cardiovascular risk and the progression of atherosclerosis, we compared the studied parameters in the groups with “isolated” hypertension and a combination of hypertension and CHD (Table 2).

As can be seen from table 2, the addition of coronary heart disease to stage II AH was accompanied by a significant decrease in the activity of NO-synthases (namely, total and inducible) compared with the

group with stage II AH without CHD and a decrease in the level of S-nitrosothiols ($p<0.05$ compared with the control group and patients with a burdened heredity). The detected changes indicate a progressive impairment of endothelial function with inhibition of NO-synthesizing systems and a decrease in the amount of NO in physiologically active depots in the association of hypertension with CHD. The level of ET-1 and vWF activity in patients with AH and CHD remained practically at the same level as in patients with AH without CHD, not significantly different between these groups ($p> 0.05$).

Table 2

Indicators of biochemical markers of endothelial dysfunction in patients with stage II hypertension, depending on the presence of concomitant coronary heart disease

Indicators.	Patients with stage II AH in general (n=231)	Stage II AH without CHD (n=110)	Stage II AH with CHD (n=121)	P
NOS total, pmol/min.*mg protein	1.94* (1.81; 2.17)	2.00* (1.84; 2.22)	1.91* (1.73; 2.11)	0.005
e-NOS, pmol/min.*mg protein	0.86* (0.72; 1.09)	0.88* (0.72; 1.06)	0.86* (0.71; 1.09)	ns
i-NOS, pmol/min.*mg protein	1.08* (0.90; 1.19)	1.11* (0.98; 1.21)	1.06* (0.86; 1.15)	0.027
RS-NO, mmol/l	0.38* (0.23; 0.51)	0.39* (0.21; 0.53)	0.38*^ (0.25; 0.49)	ns
ET-1, pg/ml	12.38*^ (9.48; 22.35)	13.18*^ (9.74; 21.49)	13.67*^ (9.48; 22.35)	ns
vWF, %.	132.0* (98.6; 147.0)	132.4* (97.4; 147.2)	130.8* (98.6; 146.3)	ns

Notes: * – the probability of differences in indicators in relation to the control group ($p<0.001$); ^ – the probability of differences in indicators in relation to the group with a burdened heredity for hypertension ($p<0.05$); P – the probability of differences between the groups of patients with "isolated" AH and a combination of AH and CHD; ns – no significant difference ($p>0.05$).

The study found that endothelial dysfunction occurs in AH, which is evidenced not only by an increase in the vasoconstrictor and thrombogenic potentials of the vascular wall, but also by a decrease in its vasodilatory function, as reported by other researchers [4, 6, 7].

The activity of NOS is regulated by the mechanism of negative correlation with the level of NO: with a lack of synthesized NO, the activity of NO-synthases increases. However, an increase in the activity of i-NOS occurs in pathological conditions characterized by the release of i-NOS from activated macrophages and its synthesis under the influence of cytokines and other biologically active substances [10]. Thus, a significant increase in i-NOS activity in patients with hypertension can be interpreted as a response to NO deficiency, on the one hand, and as an indicator of increased activity of various biological substances and manifestation of endothelial dysfunction in hypertension, on the other hand [1].

It is known that with prolonged maintenance of elevated plasma NO levels, the efficiency of its deposition increases and, on the contrary, decreases in NO-deficient states [1, 10]. Thus, our study revealed direct correlations between the level of RS-NO and the activity of e-NOS and total NOS ($r=0.48$, $p<0.0001$ and $r=0.40$, $p<0.0001$, respectively). That is, insufficient amount of NO in the endothelium stimulates the activity of e-NOS, a significant increase in the activity of which, in turn, increases not only the production of NO but also its binding to thiols, thereby leading to an increase in the level of RS-NO in the blood serum [1].

An interesting fact is the detection of impaired functional state of the vascular endothelium in practically healthy individuals with a burdened heredity for hypertension, which may indicate a certain genetic determinism of endothelial dysfunction, as well as the primary nature of its development in AH [11, 12]. These changes may to some extent confirm the opinions of other authors that the ability to deposit NO, as well as the capacity of NO-synthesizing systems, are genetically determined [10].

The level of ET-1 in the group with hereditary hypertension, although not reaching the level of ET-1 in patients with stage II hypertension, was significantly higher than the control values. The detected changes reflect an increase in the activity and tension of humoral regulators of endothelial function in people with hereditary hypertension even before the increase in blood pressure and clinical manifestations of AH [12].

Thus, the data obtained from the study indicate that in people with hereditary hypertension, even before the onset and formation of AH, changes in the functional state of the vascular endothelium occur with the development of its dysfunction, which gives reason to think about their genetic predisposition. At the same time, most biochemical markers of endothelial dysfunction in such patients do not differ significantly from those of patients with established stage II hypertension, with the exception of endothelin-1, for which such a difference was established, and RS-NO, for which the difference in levels tended to be

significant. The imbalance of humoral markers of the functional state of the endothelium in patients with genetically "programmed" hypertension reflects the common mechanisms of endothelial dysfunction and hypertension and that these phenomena are links in a single pathological process that leads to the development and progression of hypertension. That is why determination of endothelial dysfunction indicators can help in stratifying the risk of cardiovascular complications not only in patients with a diagnosis of hypertension, but also in practically healthy individuals with a hereditary predisposition to it.

Conclusion

An increase in biochemical markers of endothelial dysfunction was detected in healthy people with a burdened heredity for hypertension. The values of it significantly exceeded the levels in the control group of healthy subjects, and practically did not differ (except for ET-1 levels and a tendency to a significant difference in RS-NO levels) from the values in patients with stage II hypertension with and without coronary heart disease.

The increase in the enzymatic activity of total NO-synthase, mainly due to i-NOS, and the decrease in the level of S-nitrosothiols with an increase in the level of ET-1 indicate the predominance of vasoconstrictor reactions of the endothelium in patients with AH.

The detected abnormalities in the functional state of the vascular endothelium in individuals with a burdened heredity for hypertension may indicate that endothelial dysfunction in people with predisposed hypertension develops even before the increase in blood pressure and is genetically determined.

Further deterioration of endothelial dysfunction in comorbidity of AH with CHD was found, which was manifested by a significant ($p<0.05$) increase in the tension of the NO-synthase system, mainly due to inducible NO-synthase.

Further research in this area will improve cardiovascular risk stratification, early diagnosis of vascular endothelial dysfunction in individuals with hereditary hypertension and patients with uncomplicated AH in order to initiate or intensify endothelium-protective therapy and improve the prognosis of such patients.

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