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ENDOTHELIAL FUNCTIONAL STATE IN PREGNANT WOMEN WITH PREECLAMPSIA

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Summary. *The article presents the results of the study of endothelium vasodilation by reactive hyperemia and data on the content of nitric oxide in terms of its stable metabolites (nitrite-, nitrate-anions, LMNT) in the blood plasma of pregnant women with preeclampsia compared to healthy pregnant women who do not have gestational complications. The findings suggest that the presence of endothelial dysfunction in preeclampsia, which is one of the pathogenetic components of the development of this pregnancy complication.*

Key words: *pregnancy, preeclampsia, endothelial dysfunction, endothelium vasodilation, nitric oxide.*

Introduction. Preeclampsia is one of the main causes of maternal and perinatal morbidity and mortality. According to different authors its incidence is 12-30% in developing countries and in developed countries this figure is lower giving the hope for effective influence on the outcome of preeclamptic pregnancy [1, 2]. Despite the numerous studies on this issue, the search for answers to the certain unresolved issues of pathogenesis in orderto develop an effective management of preeclamptic pregnancy and prevent its complications, is still continuing.

Modern studies have shown that in preeclampsia there is defective cytotrophoblast cell invasion into the uterine spiral arterial wall providing maintenance of the vascular wall structure with smooth muscle fibers preserved [2, 3]. The result is the preservation of the uterine spiral arteries sensitivity to vasoconstriction. Local placental ischemia developed leads to impaired endothelial regulation of vascular tone and the development of hypertension. Complex changes in the functional state of the endothelium, which is one of the main pathogenesis of preeclampsia, is the focus of research because injury and the subsequent development of endothelial dysfunction cause major manifestations of preeclampsia, hypertension, proteinuria, edema, utero-placental insufficiency, changes in the blood coagulation. As a result microcirculation of vital organs impair with the development of multiple organ failure.

According to the modern concepts vascular endothelium is a large active neuroendocrine organ that diffusely spread in all tissues. Its main function isregulation of vascular penetration and tone,and it is involved in hemostasis, vascular remodeling, control of nonspecific inflammatory reactions, immune and enzyme activity [5].

The most important modulator of most endothelial functions is nitric oxide, the most powerful vasodilator [5]. It is produced in endothelial cells from L-arginine in the presence of oxygen by the enzyme NO-synthase [6]. Apart of this mechanism,there is nitrite reductase regeneration system of nitric oxide, its role in creases in oxygen deficiency. The average lifeof nitric oxide is only a few seconds; therefore it autoregulates blood flowlocally. This L-arginine/nitric oxide system provides the leading role ofvasoregulation duringgestation [4, 8]. It is known that normal pregnancy is accompanied by increased activity of the renin-angiotensin-aldosterone system (RAAS), which increases plasma and total fluidvolume in the body, while total peripheral resistance (TPR) and blood pressure (BP)declinein the background of the RAAS activation, which may be associated with decreased vasoconstrictor response to peptides and aminesin pregnancy. This, in turn, is due to the intensification of the L-arginine/nitric oxide system and is associated with increased hormone levels in pregnancy [9-10]. At this stage it is endothelial dysfunction that is increasingly associated with pregnancy complications such as preeclampsia and placental insufficiency [3, 10].

The reduced placental blood flow leading to the placental ischemia includes compensatory mechanisms to

restore its perfusion. Vasopressor factors secreted by ischemic placenta increase endothelial dysfunction; this entails progression of hypertension and preeclampsia in pregnant development, which closes the vicious circle [3,8]. Thus, detection of endothelial dysfunction as a predictor of preeclampsia and placental insufficiency in terms of nitric oxide could be very promising, but molecule instability drastically limits the use of this method. The study of stable nitric oxide metabolites in plasma and urine can be used routinely in the clinic due to the extremely high requirements for preparation of the patient in the study. Other laboratory methods to assess endothelial function (determination of plasma desquamative endotheliocytes, endothelin, prostacyclin, fibronectin, cytokines, etc.) do not have a high degree of specificity and diagnostic value as most markers are formed not only in the endothelium, but also in other tissues [6].

As an alternative definition of biochemical markers in clinical practice the instrumental diagnostic methods used to assess endothelial function, which in turn are divided into invasive and non-invasive methods, were proposed [5]. Of the non-invasive methods the most commonly used test in the worldis reactive hyperemia of brachial (femoral) artery using high resolution ultrasound, which was proposed in 1992 by DS Celemajer et al. [7]. The method is based on the ability of the endothelium to release nitric oxide and other vasodilators in response to shear stress (reactive hyperemia). With intact endothelial function, this leads to flow-dependent dilation artery. To achieve post-occlusion reactive hyperemia, the 5-minutes phygmomanometer cuff compression with the artery diameter measurement before occlusion and in the first minute of decompression using linear ultrasonic sensor was proposed. The endothelium-dependent vasodilation (EDV) is calculated as the relative increase in the diameter of blood vessels and as a norm is 10% or more. EDVvalue reduces in endothelial dysfunction. Choice of the brachial or femoral arteries for to test is not accidental, because these vessels are situated on the surface suitable for visualization and have an ideal diameter, since change in the diameter of smaller vessels is harder to be registered, and dilation of larger arteries is negligible. In 2002 the current international guidelines for EDV ultrasound were published, and this method is considered to be safe and highly recognized in the assessment of severity of endothelial dysfunction [5].

The purpose of this study is to assess the functional state of the endothelium in pregnant women with preeclampsia by investigation of endothelium-dependent vasodilation in the brachial artery reactive hyperemia test and by definition of nitric oxide levels (stable metabolites, nitrite anion, nitrate anion and low molecular nitrosothiols).

Materials and methods.

The study involved 118 women of reproductive age (19-37 y) in the third trimester of pregnancy with preeclampsia without somatic disease (group 1). All women had clinical and laboratory examination in accordance with the order of Ministry of Health of Ukraine for pregnant women with hypertensive disorders. The exclusion criteria for women with preeclampsia were somatic pathology (diabetes mellitus, chronic hypertension, renal disease, connective tissue disease, infectious disease, heart disease), treatment with aspirin, non-steroidal anti-inflammatory drugs, antibiotics and complications of pregnancy (chorioamnionitis, polyhydramnios, multiple pregnancy). As a control, 30 pregnant women with uncomplicated pregnancies at the same gestational age (group 2) were examined. Groups are representative by the age, physical and gynecological pathology.

In addition to the necessary clinical and laboratory investigations in all pregnant after obtaining informed consent the functional studies of endothelial function based on the results of endothelium-dependent vasodilation (EDV) and blood sampling were conducted to determine the level of nitric oxide by its stable metabolites, nitrite and nitrate anions, low molecular nitrosothiols.

EDV was evaluated by triplex ultrasound using 7.5-12 MHz ALOKA SSD-1700 convex transducerby measuring the diameter of the brachial artery before and after (30, 60, 90, 120 s) 5-minute occlusion with a cuff sphygmomanometer with pressure, 50 mm Hg. higher than the systolic pressure. EDV was calculated as the percentage of increase in brachial artery diameter after decompression in relation to the baseline.

The stable metabolites of nitric oxide was determined by Green method, nitrite anion (N2O-) was determined colorimetrically using Gris reagent (Green LCet al., 1982), nitrate anion (NO3-) – colorimetrically using brucine reagent (Bank NR, 1993). The number of nitrite and nitrate anions was determined using calibrated curvesconstructed according to sodium nitrite and nitrate. The content of low molecular nitrosothiols (LMNT) was determined by Saville (Gergel D., Cederbaum AI, 1991) using mercury nitrate for S-NObond hydrolysisof protein-free sample extracts.

The results of laboratory and instrumental investigations were processed by mathematical and statistical analysis with calculation of means (M+m), Student's test and confidence value using electron table processing "Excel 7.0 for Windows XP".

Results and discussion. All women were in the active reproductive age, the average age had no significant difference in groups and was 27.6 ± 1.3 for group 1;26.5 ± 1.2 for group 2, (p – 0.05).The mean systolic blood pressure (SBP) in patients of this group with mild preeclampsia was 134.2 + 3.5 mm Hg, and moderate preeclampsia was 158.8 + 5.3 mm Hg. The mean diastolic blood pressure (DBP) in women with mild preeclampsia was 75.1 + 1.6 mm Hg, and with moderate preeclampsia was 93.4 + 2.1 mm Hg. 67 (56.8%) women developed preeclampsia with dominated hypertensive syndrome, 35 (29.7%) had edema syndrome, 16 (13.6%) had classic preeclampsia triad (hypertension, edema and proteinuria). In group 2 (control) the average SBP was 121.3 ± 2.1 mm Hg, and DBP was 72.3 ± 2.0 mm Hg.

The results of endothelial assessment the pregnant subjects are shown in Table 1.

The study of the average diameter of the brachial artery at rest in healthy pregnant women (group 2) showed that its value was 3.73 ± 0.30 mm and after occlusion test it was 4.20 ± 0.40 mm. The dilation in the reactive hyperemia background led to an increase in the diameter of 15.3%. The

Table 1.

Endothelium-dependent vasodilation values in the studied pregnant women

Values		Preeclamptic pregnancy n - 118	Control n - 30
Artery diameter (mm)	Before test	3.89±0.20	3.73±0.80
	After test	3.75±0.40	4.30±0.40
	%	3.6%	15.3%
Blood flow rate (m/s)	Before test	0.50±0.01	0.48±0.02
	After test	0.63±0.02*	0.78±0.01*,**
	%	26,0%	62.5%

Note: * – specified probability p<0.05 compared to control, **- before and after test

Table 2.

Stable metabolites of nitric oxide and LMNT in plasma of the studied pregnant women, M+m

Group	Values		
	NO_2 pmol/ml	NO_3 nmol/ml	LMNT pmol/ml
Group 1 (preeclampsia) n - 118	88.3 ± 4.7*	189.5 ± 8.4*	2170.8 ± 19.6*
Group 2 (control) n - 30	114.8 ± 12.3	217.0 ± 10.5	2822.0 ± 22.7

Note: probability p<0.05 comparedto control

normal reaction artery in response to reactive hyperemia is 10.0-13.0% of the baseline diameter.

Thus, the healthy pregnant had the adequate vascular response, which showed the balanced state between lumen-narrow in gpower of tonicblood vessel state and tensile blood flow.

In the group of women with preeclampsia the diameter changes were the smallest, there were no significant differences between the largest vessel diameter before and after the test. In addition, it should be noted that the average diameter of the brachial artery in women with preeclampsia before the test was 3.89 ± 0.20 mm and after occlusion test it was 3.75 ± 0.40 mm that is even lower. The percentage increase was only 3.6%, which was almost 5 times lower than in the control group of pregnant women.

Doppler study showed that the average blood flow rate in a brachialis in healthy pregnant women was 0.48 ± 0.02 m/s. It increased to 0.78 m/s in reactive hyperemia. The value increase was 62.5% of the baseline, indicating that the vascular effect on the function test was positive.

In pregnant women with preeclampsia the increase in the blood flow rate was almost twice lower than in the control group and was 0.50 m/s before test and 0.63 m/s after the test. The value increase was 26.8%, indicating inadequate vascular effect in response to the functional tests. Thus, according to the results of the brachial artery reactive hyperemia test suggest endothelial dysfunction in pregnant women with preeclampsia.

The absence of endothelium-dependent vasodilation response to physiological stimuli may mean reduced production or increased inactivation of nitric oxide [5,6]. To determine this suggestion the nitrogen oxide levels were evaluated for its stable metabolites and low nitrosothiols (Table 2). The results showed that women with preeclampsia had a decrease in levels of stable metabolites of nitric oxide and increased LMNT.

The results showed that the development of preeclampsia is accompanied with nitric oxide deficiency providing vasodilation. Thus, the results indicate the presence of endothelial dysfunction in pregnant women with preeclampsia due to low vasodilators (nitric oxide), as evidenced by low levels of stable metabolites of nitric oxide in plasma compared to the control, and the results of the reactive hyperemia tests.

Conclusions

1. The development of preeclampsia is accompanied with endothelial dysfunction caused by decrease in active vasodilators (nitric oxide) in the maternal body.

2. Using endothelium-dependence vasodilation test in pregnant women with risk factors for preeclampsia is an effective non-invasive method for determining the functional state of the endothelium.

Reviewer: Corresponding Member NAMS Ukraine, professor B.M. Ventskovskiy

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ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ЕНДОТЕЛИЯ У БЕРЕМЕННЫХ С ПРЕЭКЛАМПСИЕЮ

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Резюме. В статье представлены результати исследований эндотелийзависимой вазодилятации по пробе с реактивной гиперемией и данные о содержании оксида азота по уровню его стабильных метаболитов (нитрит-, нитрат-антионов, НМНТ) в плазме крови у беременных с преэклампсией в сравнении со здоровыми беременными, не имеющими гестационных осложнений. Полученные данные свидетельствуют о наличии эндотелиальной дисфункции при преэклампсии, являющейся одним из патогенетических звеньев развития данного осложнения беременности.

Ключевые слова: беременность, преэклапсия, эндотелиальная дисфункция, эндотелийзависимая вазодилятатация, оксид азота.

ФУНКЦІОНАЛЬНИЙ СТАН ЕНДОТЕЛІЮ У ВАГІТНИХ З ПРЕЕКЛАМПСІЄЮ

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Резюме. У статті представлені результати досліджень ендотелійзалежної вазодилятації по пробі з реактивною гіперемією і дані про вміст оксиду азоту за рівнем його стабільних метаболітів (нітрит, нітрат-антіонов, НМНТ) у плазмі крові у вагітних з прееклампсією в порівнянні зі здоровими вагітними, не мають гестаційних ускладнень. Отримані дані свідчать про наявність ендотеліальної дисфункції при прееклампсії, що є одним з патогенетичних ланок розвитку даного ускладнення вагітності.

Ключові слова: вагітність, прееклапсія, ендотеліальна дисфункція, ендотелійзалежна вазоділятатція, оксид азоту.