

Markers of Endothelial Dysfunction as Criteria for Differential Diagnosis of Hypertensive Disorders in Pregnant Women

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The aim of the investigation was to study indicators of microvasculature oxygenation and markers of endothelial dysfunction in the peripheral blood of pregnant women with various hypertensive disorders and on the basis of the resulting findings to suggest new diagnostic criteria for this pathology.

Materials and Methods. The study involved 153 women at 22–37 weeks gestation: 41 women with chronic arterial hypertension (CAH), 22 women with CAH and secondary preeclampsia (PE), 50 women with PE, 40 women with normal blood pressure. The level of tissue oxygenation (SO₂) was estimated by test with ischemia/reperfusion (Spectrotest, Russia). Desquamated endotheliocytes (DE), total nitrates and nitrites (NOx) were determined in the peripheral blood.

Results. All kinds of hypertensive disorders in pregnant women are accompanied by the development of endothelial dysfunction as evidenced by the change in the level of tissue oxygenation, increase in DE and NOx content in the blood. The extent of endothelial function impairment depends on PE severity.

Conclusion. Changes in endothelium-dependent vascular response found as the result of test with ischemia/reperfusion and the content of venous endothelial cells in pregnant women with hypertensive disorders may serve as diagnostic criteria of hypertensive disorders in pregnancy, giving the possibility to choose tactics of patient management and to start well-timed adequate therapy.

Key words: preeclampsia; hypertension in pregnancy; endothelial dysfunction; tissue oxygenation; test with ischemia/reperfusion.

Hypertensive disorders in pregnant women remain an acute problem in modern medicine, being one of the leading causes of maternal and perinatal mortality. According to the World Health Organization findings, the incidence of hypertensive syndrome in pregnant women is 4–8%, with severe preeclampsia (PE) being diagnosed in 5 out of 1,000 pregnant women and eclampsia in 5 out of 10,000 women [1]. In Russian Federation arterial pressure (AP) higher than 140/90 mm Hg is revealed in 5–20% of pregnant women, in some regions this number amounts to 29% [2].

Arterial hypertension (AH) in pregnancy is a symptom of numerous conditions that involve chronic arterial hypertension (CAH), CAH with secondary proteinuria, PE. Variety of clinical forms of this pathology causes difficulties in making a differential diagnosis of hypertension in pregnancy. Difficulties in diagnosis

may occur due to physiological AP reduction in the 1st trimester, undulating course of CAH, its exacerbation at the end of pregnancy [3]. AP diagnosis in pregnancy is made on the basis of at least two elevated AP values; in a dubious case 24-hour AP monitoring is recommended, which is performed in far too few patients as it requires a hospital stay that is not always possible. In presence of PE it is necessary to ensure timely delivery since termination of pregnancy is the only etiologic method of treatment for this complication. It should be noted that pregnant women with CAH have more favorable prognosis.

In pathogenesis of hypertensive disorders a great importance is attributed to endothelial dysfunction along with immunological factors [4, 5]. According to one of the theories, hypertension development in pregnancy is caused by systemic inflammatory reaction in which

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the most relevant part is damage to the endothelium and its function impairment [6]. Endothelial dysfunction in hypertensive disorders is known to manifest itself by changed vessel wall microcirculation and elevated adhesiveness of vascular bed [7]. Generalized vascular spasm and capillary blood flow impairment, which occurs in CAH with PE, leads to circulatory hypoxia [8]. Intravital study of the functional state of vascular endothelium in pregnancy is extremely complicated. The data on impaired endothelium-dependent vasodilation in hypertensive pregnant women is still insufficient.

Scientific literature of recent years offers the following indexes as differential diagnostic criteria for hypertensive disorders in pregnant women: echocardiography findings, hemodynamics type, glomerular filtration rate [9], microalbuminuria stage [10], the level of angiogenic factors (VEGF, PIGF, sFlt-1, sEng) [11], cell-free total and fetal DNA in blood plasma [12], the number of neurospecific proteins in the placenta tissue (NSE и GFAP) [13] and adhesion molecules by peripheral phagocytes (CD49b, CD11b, CD51, CD99) [14, 15]. However, labor intensity, technical complexity and rather high cost of these methods limit their wide application in practice.

Therefore, finding additional diagnostic criteria for hypertensive disorders in pregnant women is still vitally important for determining timely treatment and adequate tactics of patient management.

The aim of the investigation was to study the indexes of microcirculatory system oxygenation and markers of endothelial dysfunction in the peripheral blood of pregnant women with various hypertensive disorders and based on the resulting findings to suggest new diagnostic criteria for this pathology.

Materials and Methods. One hundred and fifty-three women at 22–37 weeks gestation were examined at the Obstetric Clinic of Ivanovo Research Institute of Motherhood and Childhood named after V.N. Gorodkov. Among them 113 women with various hypertensive disorders were divided into the following groups: group 1 (n=41) included women with CAH (ICD-X code O10.0), group 2 (n=22) consisted of women with CAH and secondary PE (ICD-X code O11), group 3 (n=50) included women with moderate and severe PE (ICD-X code O14.0, O14.1). The control group was composed of 40 pregnant women with normal AP indexes.

The study complies with the Declaration of Helsinki (adopted in June 1964 (Helsinki, Finland) and revised in October 2000 (Edinburgh, Scotland)) and was approved by the Ethics Committee of Ivanovo Research Institute of Motherhood and Childhood named after V.N. Gorodkov, Ministry of Health of Russia. All the patients gave informed consent to participate in the study.

Exclusion criteria were secondary (symptomatic) AH (ICD-X codes O10.1, O10.2, O10.3, O10.4), acute and chronic inflammatory diseases in exacerbation phase, allergic reactions at the time of examination, tumors

of different localization, systemic connective tissue diseases, chronic renal insufficiency.

Mean age of pregnant women with CAH (34.1 ± 0.74 years) and those with CAH and secondary PE (32.9 ± 0.90 years) was statistically significantly older than in the control group (27.6 ± 0.6 years) and in the group of women with PE (28.4 ± 0.6 years) irrespective of its severity ($p=0.001$ in all cases).

Women with hypertensive disorders showed higher incidence rate of respiratory and urinary tract diseases in the past history ($p=0.001$ in both cases) as compared to the control group. Inherited predisposition to hypertension, hypertensive disorders in previous pregnancies were also more frequent in women from the main group ($p=0.01$ in both cases). Gestation complications such as placental insufficiency with uterine-placental and/or fetal-placental misperfusion were more common in patients of groups 2 and 3 ($p=0.001$ as compared to group 1 and the control group in all cases). The incidence rate of preterm delivery was also statistically significantly higher in women of groups 2 and 3 as compared to group 1 and the control group ($p=0.001$ in all cases), with PE women having the shortest gestational term by the time of delivery (32.7 ± 0.4 weeks) ($p=0.001$ compared to CAH). Pregnant women with hypertensive disorders unlike the control group more often delivered babies in a state of asphyxia, with perinatal pathology, their babies were more often transported from delivery room to the neonatal resuscitation unit in need of follow-up care ($p=0.001$ in all cases).

To estimate the level of microcirculatory system oxygenation in the forearm tissue functional test with ischemia/reperfusion was used. The study was performed with noninvasive spectrophotometric device for measuring volumetric capillary blood content in the soft biological tissue, Spectrotest (SPE "Cyclone-Test", Russia).

The test with ischemia/reperfusion was performed using standard methods. Measurements were taken in the lower third of the inner surface of the forearm. The patient was in a sitting position, with her forearm placed at the heart level. The air temperature amounted to $+20 \dots 22^\circ\text{C}$. Initial AP was measured 10 min prior to the examination. Before testing a standard blood pressure cuff was applied on the pregnant woman's upper arm without pressurization. The initial oxygenation level was determined using basic test during 180 s. Additional positive pressure exceeding the initial systolic pressure by 40–50 mm Hg was generated with the blood pressure cuff. Along with the continuous recording of indexes arterial occlusion was maintained for 180 s. This time being over, the pressure in the cuff was dropped while recording was continued for 180 s more. Total time of index recording amounted to 540 s. Apart from the initial level, tissue oxygenation in reperfusion phase was estimated: the maximal value of forearm tissue

oxygenation after recovery of arterial blood flow (after compression) and the final oxygenation level that was calculated as mean oxygenation index for the last 10 s of examination, the ratio of the final oxygenation level to the initial one was determined as well.

The number of circulating endothelial cells (desquamated endotheliocytes (DE)) was determined in the peripheral blood (cells/ μ l) according to the method of Hladovec (1978) using MIKMED-1 apparatus (Russia) for phase contrast microscopy and counting endothelial cells in the Goryayev chamber. Total nitrates and nitrites (NO_x) were determined by converting nitrates to nitrites in presence of vanadium chloride (Miranda, 2001) using Solar PV 1251C spectrophotometer (JSC "Solar", Belarus Republic).

Statistical analysis was carried out using variation statistics methods with applied licensed software package Microsoft Office 2010, Statistica for Windows 6.0, MedCalc v7.4.4.1. Quantitative values were expressed as mean and standard error of mean ($M \pm m$) (normal distribution) and as a median with the 25th and 75th percentiles (Me [25; 75]) (if distribution was different from normal). Significance of differences between the indexes was evaluated using Student t-criterion and Mann–Whitney U-test (significance level $p < 0.05$ was regarded as statistically significant). To evaluate diagnostic significance of the studied indexes ROC-analysis was used.

Results and Discussion. The performed investigation showed (See the Table) that in pregnant women of the control group in conditions of reperfusion forearm tissue oxygenation level increased insignificantly ($p > 0.05$) compared to the initial tissue oxygenation indexes before the occlusion test (mean 0.69 ± 0.03 relative units) but finally it was statistically significantly lower than the initial values: 0.65 ± 0.02 relative units ($p = 0.001$).

In women with hypertensive disorders initial indexes of tissue oxygenation were no statistically significantly

different from the control group ($p > 0.05$). However, in the reperfusion phase oxygenation level grew significantly higher than the initial value (to 0.75 ± 0.01 relative units; $p = 0.01$). The final oxygenation level in this group amounted to 0.67 ± 0.01 relative units, which was statistically significantly lower than the initial values ($p = 0.001$) and similar to the control group ($p > 0.05$).

The ratio of the final oxygenation level to the initial one in the main and control groups had no statistically significant differences.

Depending on the type of hypertensive disorder, the response to test with ischemia/reperfusion was various. The initial SO₂ level was similar in all the groups with hypertensive disorders and the control, which was confirmed by the data obtained in the earlier studies [16]. PE women showed maximal growth of oxygenation level in the reperfusion phase compared to the initial values ($p = 0.001$). In the group with CAH and PE oxygenation index remained unchanged in the reperfusion phase ($p > 0.05$), which can be interpreted as impairment of adaptive capabilities in case of PE secondary to the existing hypertension wherein long-term mechanisms of pathological adaptation are formed [17].

Vascular function monitoring during the test with ischemia/reperfusion is based on the idea that change in oxygenation level during and after the ischemic exposure reflects changes in the blood flow. Significant decrease in the final oxygenation level was noted in all the groups, but in women with CAH this index was minimal. Significant reduction of the final SO₂ value in this group indicates the presence of marked peripheral vasospasm and microcirculation process impairment associated with hypovolemia, which agrees well with the findings of other authors [18].

The ratio of the final oxygenation level to the initial one in women with CAH was statistically significantly lower than in the control group and groups with hypertensive disorders specific for pregnancy ($p = 0.001$ in all cases).

Tissue oxygenation indexes in different phases of

The level of forearm tissue oxygenation (SO₂) in pregnant women during the test with ischemia/reperfusion ($M \pm m$)

SO ₂ value (relative units)	Control (n=40)	Main group (n=113)	CAH (n=41)	CAH with PE (n=22)	PE (n=50)
Initial	0.69 ± 0.03	0.72 ± 0.01	0.72 ± 0.02	0.73 ± 0.03	0.73 ± 0.02
In reperfusion phase	0.71 ± 0.02	$0.75 \pm 0.01^{**}$	$0.74 \pm 0.02^*$	0.73 ± 0.03	$0.77 \pm 0.02^{***}$
Final	$0.65 \pm 0.02^{***}$	$0.67 \pm 0.01^{***}$	$0.63 \pm 0.03^{***}$	$0.69 \pm 0.03^{***}$	$0.69 \pm 0.02^{***v}$
Ratio of final level to initial	0.94 ± 0.02	0.92 ± 0.01	$0.86 \pm 0.02^{**}$	0.95 ± 0.01^{vv}	0.95 ± 0.01^{vvv}

Note. CAH: chronic arterial hypertension; PE: preeclampsia. Statistically significant difference between the values and the initial index: * $p = 0.02$; ** $p = 0.01$; *** $p = 0.001$. Statistically significant difference between the values and the control group parameters: + $p = 0.04$; ++ $p = 0.001$. Statistically significant difference between the values and CAH group parameters: v $p = 0.04$; vv $p = 0.01$; vvv $p = 0.001$.

investigation in PE group did not depend on its severity ($p>0.05$).

The endothelial function was also estimated by determining the number of DE and NOx in peripheral blood. It was established that in all women with hypertensive disorders irrespective of their genesis DE and NOx levels were statistically significantly higher (13.0 [8.0; 18.0] and 70.0 [63.0; 83.0] $\mu\text{mol/L}$, respectively) compared to the control group (5.0 [4.0; 7.0] and 67.0 [62.5; 71.3] $\mu\text{mol/L}$; $p=0.001$ and $p=0.04$, respectively). Notably, statistically significant differences in NOx content were found only in PE group ($p=0.02$) as compared to the control, while the number of circulating endothelial cells significantly increased in all groups with hypertensive disorders ($p=0.01$ in all cases). Moreover, the level of circulating endothelial cells in women with severe PE was significantly higher than in those with moderate PE: 17.0 [15.25; 22.5] cells/ μl against 14.0 [8.0; 16.0] cells/ μl ($p=0.01$).

DE are known to be specific markers of damage to inner vessel bed. Increased DE content in the blood of pregnant women with hypertensive disorders reflects the very process of endotheliosis induced by the influence of endotoxins, superoxide radicals, homocysteine, histamine and other damaging factors [19]. Our findings are consistent with the studies revealing the fact that the level of circulating endotheliocytes increases in pregnant women with hypertensive disorders [20].

Another marker of endothelial dysfunction, NO and its metabolites, was also increased in pregnant women from the main group which might indicate activation of compensatory mechanisms as a result of hypoxia developed in AH [21]. Literature provides rather contradictory data on the given marker: some

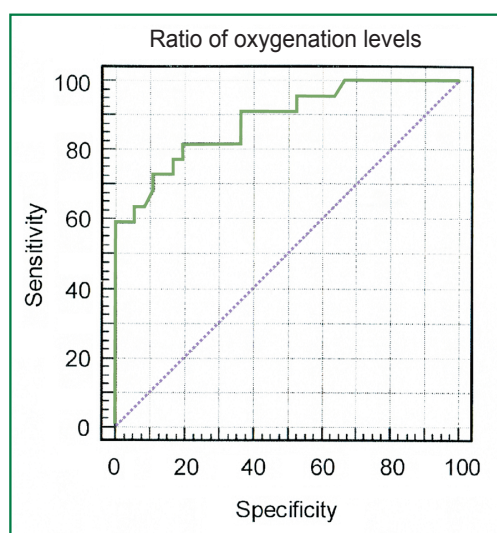
researchers point out the decrease in the level of NO and its metabolites in peripheral blood [22], others have revealed no differences in NO-synthetase activity and NO production in AH and physiological pregnancy [23].

Correlational analysis performed in women with CAH revealed inverse relationship between increased content of nitrogen oxide metabolites and the level of forearm tissue oxygenation, initial ($R=-0.44$; $p=0.02$) and final ($R=-0.46$; $p=0.01$). According to our data, in other groups no correlations between biochemical markers of endothelial dysfunction and forearm tissue oxygenation indexes were established.

In order to determine the most sensitive and specific criteria for diagnosing various forms of hypertensive disorders in pregnancy there was performed ROC-analysis, which showed that certain values of the studied indexes may serve as additional diagnostic criteria for various forms of hypertension in pregnant women. For diagnosing PE the most informative criterion appeared to be the level of forearm tissue oxygenation, determined in reperfusion phase (borderline value was more than 0.72 relative units; sensitivity was 72.0%; specificity — 55.0%; AUC area under the ROC-curve — 0.607). DE number in peripheral blood was the most accurate criterion for determining PE severity (borderline value was 14 cells/ μl ; sensitivity — 77.8%; specificity — 70.6%; AUC area — 0.778). Diagnostic criterion for secondary PE in pregnant women with CAH was the ratio of the initial tissue oxygenation level to the final one (borderline value was 0.93 relative units; sensitivity was 81.8%; specificity — 80.5%; AUC area — 0.811 (See the Figure). There was submitted patent application No.2015137369 for elaboration of the given criteria dated 01.09.2015.

The obtained findings allowed the authors to develop an algorithm for diagnosing various forms of hypertensive disorders in pregnant women. In order to determine the form of hypertension in pregnant women in the second half of gestation the authors suggest carrying out a functional test with ischemia/reperfusion. In case of tissue oxygenation level in reperfusion phase being higher than 0.72 relative units PE should be diagnosed; with the ratio of the final oxygenation level to the initial one higher or equal to 0.93 relative units it is possible to establish PE secondary to CAH; less than 0.93 relative units is characteristic of CAH. To make PE severity more accurate it is recommended to determine DE in peripheral blood: their number being more than 14 cells/ μl indicates severe PE.

Conclusion. All types of hypertensive disorders in pregnancy are accompanied by development of endothelial dysfunction, which is confirmed by the change in tissue oxygenation level, increased number of desquamated endotheliocytes and blood content of total nitrates and nitrites. Intensity of endothelial function impairment depends on preeclampsia severity. Changes in endothelium-dependent vascular response found



Characteristics of diagnostic values of the ratio of the final forearm tissue oxygenation level to the initial one during the test with ischemia/reperfusion according to ROC-analysis data

in pregnant women with hypertensive disorders as the result of test with ischemia/reperfusion and the content of venous endothelial cells may serve as diagnostic criteria of hypertensive disorders in pregnancy, giving the possibility to choose tactics of patient management and to start well-timed adequate therapy.

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Conflicts of Interest. The authors have no conflicts of interest to disclose.

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