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 etiotropic 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...
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, PI GF, 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 О10.0), group 2 (n=22) consisted of women with CAH and secondary PE (ICD-X code О11), group 3 (n=50) included women with moderate and severe PE (ICD-X code О14.0, О14.1). 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 О10.1, О10.2, О10.3, О10.4), 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 ... 22°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
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

<table>
<thead>
<tr>
<th>SO2 value (relative units)</th>
<th>Control (n=40)</th>
<th>Main group (n=113)</th>
<th>CAH (n=41)</th>
<th>CAH with PE (n=22)</th>
<th>PE (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.69±0.03</td>
<td>0.72±0.01</td>
<td>0.72±0.02</td>
<td>0.73±0.03</td>
<td>0.73±02</td>
</tr>
<tr>
<td>In reperfusion phase</td>
<td>0.74±0.02</td>
<td>0.75±0.01**</td>
<td>0.74±0.02*</td>
<td>0.73±0.03</td>
<td>0.77±02***</td>
</tr>
<tr>
<td>Final</td>
<td>0.65±0.02***</td>
<td>0.67±0.01**</td>
<td>0.63±0.03***</td>
<td>0.69±0.03***</td>
<td>0.69±0.02* ***</td>
</tr>
<tr>
<td>Ratio of final level to initial</td>
<td>0.94±0.02</td>
<td>0.92±0.01</td>
<td>0.86±0.02**</td>
<td>0.95±0.01**</td>
<td>0.95±0.01***</td>
</tr>
</tbody>
</table>

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: *p=0.04; **p=0.01; ***p=0.001.
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 nitrates. Intensity of endothelial function impairment depends on preeclampsia severity. Changes in endothelium-dependent vascular response found.
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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.

References