EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Lipid-Protein Complexes in Erythrocyte Membrane in Late Gestosis

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Gestosis is characterized by increased rigidity of the surface and deep layers of erythrocyte membranes, impaired protein-lipid relationships, and imbalance between LPO and the antioxidant defense system. These changes determine the prognosis and complications of this condition.

Key Words: *gestosis; nephropathy; lipid-protein cross-links; lipid matrix; ceruloplasmin; apotransferrin*

Pregnancy is associated with considerable metabolic changes in the body, in particular, activation of lipid metabolism. Lipids are the initial material for membrane and organelle synthesis. They determine membrane state and properties, their osmotic stability and permeability. Increased membrane viscosity can lead to conformation changes in integral proteins [2,7].

An important role in the pathogenesis of late gestosis is played by membrane disorders, in particular, decreased total lipid content due to reduced content of cholesterol and phospholipids [3,5,7], which indicates low density of lipid molecule packing and enhanced free-radical oxidation of lipids [1,4], *i.e.* conditions of oxidative stress. Processes of membrane destruction in late gestosis are responsible for disorders in intrauterine growth and development of the fetus [5,8,9].

Lipid peroxidation (LPO) processes are regulated by the antioxidant system including various compounds, specifically ceruloplasmin (CP) and apotransferrin. These copper- and iron-containing proteins bind superoxide radicals and oxidize Fe(II), the main inducer of free-radical processes [13]. Intensification of LPO can damage not only membrane phospholipid bilayer, but also induce polymerization of membrane proteins [17].

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Here we studied lipid-protein relationships in erythrocyte membranes (EM) in health and late gestosis.

MATERIALS AND METHODS

A total of 137 women at different terms of gestation were examined (67 women with gestosis and 70 with normal course of pregnancy). Control group consisted of 36 healthy women of reproductive age. LPO intensity in isolated plasma membranes was evaluated by the content of hydroperoxides and malonic dialdehyde (MDA) measured by the reaction with thiobarbituric acid; the content of ceruloplasmin and transferrin (TF) was evaluated by EPR spectroscopy and by chemiluminescent technique. Structural characteristics of EM were evaluated by changes in microviscosity and hydrophobicity and by protein exposure. Fluorescence of erythrocyte ghosts and probes in EM was measured on a Hitachi-4 spectrofluorimeter. The results were processed using Student's *t* test.

RESULTS

In normal pregnancy, the concentrations of all LPO products in the serum and EM gradually increased and peaked before labor (Table 1). The most strict correlations were found between MDA level and parame-

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ters of ordering (r=0.64, p<0.01) and hydrophobicity (r=-0.52, p<0.05). The content of hydroperoxides in erythrocytes correlated with membrane microviscosity (r=0.57, p<0.05).

The serum concentration of CP peaked at weeks 24-28 of gestation: it 2.4-fold surpassed the corresponding value in nonpregnant women (Table 1). In $^3/_4$ pregnant women this parameter varied from 3.70 to 1.74 arb. units, while in healthy nonpregnant women it varied from 1.50 to 1.74 arb. units.

A slight decrease of CP content was observed at weeks 29-32: in $^2/_3$ pregnant women CP level did not exceed 3.45 arb. units. At weeks 33-37 serum CP concentration continued to decrease, which was seen from decreased mean (Table 1) and individual values (2.90-3.20 arb. units). By weeks 38-40, CP level increased compared to the previous term (p<0.05, Table 1).

Along with CP, apo-TF plays an important role in serum antioxidant activity (AOA). This protein is converted into TF upon binding iron; TF delivers iron to cells. The concentration of TF in the blood inversely correlates with apo-TF content and serum AOA. At weeks 24-28 of pregnancy, the content of TF decreased by 20% (p<0.05), which was confirmed in individual analysis: in $^3/_4$ examinees this parameter varied from 1.7 to 1.85 arb. units, while in healthy nonpregnant women it was 2.12-2.4 arb. units. The minimum TF content was observed at weeks 33-37 (Table 1), indicating a high AOA potential. As pregnancy progressed, the studied parameters tended to increase.

The CP/TF ratio at weeks 24-28 and 33-37 was 2.8 and 3.4 times higher than in nonpregnant women (p<0.001, Table 1). Serum AOA was the highest during this period. At weeks 38-40, the CP/TF ratio was the lowest compared to the previous terms (Table 1). This decrease in CP/TF ratio can be due to elevation of serum TF concentration in full-term pregnancy. Coefficient of correlation between the CP/TF ratio and pregnancy term was -0.53 (p<0.05). Our previous studies [9] showed that by the end of the third trimester the content of LPO products in the blood increased, which was associated with pronounced dysproteinemia at the expense of CP and TF.

Despite notable activation of LPO in the serum and EM, there were no essential changes in the characteristics of the lipid matrix in pregnant women. Membrane microviscosity and hydrophobicity did not differ from those in nonpregnant women until week 37 (0.6700±0.0001 *vs.* 0.6710±0.0001 arb. units).

The concentrations of CP and TF in pregnant women with gestoses of different severity differed significantly from those in healthy nonpregnant women (Table 2).

Serum CP level in women with mild gestosis was higher than in healthy nonpregnant women (p<0.05), in $^2/_3$ pregnant women individual values varied from 3.3 to 4.7 arb. units *vs.* 2.7-4.1 arb. units in the control group. In moderate nephropathy serum CP content decreased compared to normal pregnancy (p<0.02) and mild gestosis (p<0.001).

Individual analysis showed that this parameter was 2.0-3.0 arb. units in moderate gestosis and 1.3-2.5 arb. units in severe gestosis.

Since TF level inversely correlates with serum AOA, the increase in this parameter indicates a decrease in AOA. Serum TF concentration in mild nephropathy was virtually the same as in normal pregnancy (Table 2). However, in individual analysis this parameter varied from 1.1 to 2.0 arb. units in $^2/_3$ patients with mild gestosis and from 2.00 to 2.24 arb. units in $^2/_3$ healthy pregnant women.

In moderate gestosis the content of TF increased 1.3 times compared to that in normal pregnancy (p<0.01) and 1.5 times compared to mild gestosis (p<0.001). Blood levels of TF in severe and moderate nephropathy were similar and significantly surpassed the corresponding value in mild nephropathy (p<0.001, Table 2).

The same trend was observed for individual values.

The CP/TF ratio more adequately reflects the state of proteins involved in AOA of the blood. In mild gestosis this parameter increased 1.3-fold compared to normal pregnancy (Table 2). Individual values in $^2/_3$ of examinees varied from 2.2 to 5.0 (normal 1.6 to 2.1).

In moderate nephropathy the CP/TF coefficient decreased 2.2-fold compared to that in mild nephropathy (p<0.001, Table 2). This can be due to essential decrease in CP level and increase in TF level in moderate gestosis. In pregnant women with severe nephropathy the decrease in CP/TF coefficient was the most demonstrative in the analysis of individual values: 0.96-1.44 in moderate nephropathy and 0.53-0.87 in severe condition. It can be assumed that decompen-

TABLE 1. Changes in Serum CP and TF Concentrations (Arb. Units) at Various Terms of Pregnancy ($M\pm m$)

Gestation term, weeks	CP	TF	CP/TF
24-28	3.90±0.12$^+$	1.85±0.07	2.15±0.13
29-32	3.70±0.11*	1.90±0.09	2.000±0.089
33-37	3.2±0.09**	1.27±0.08*	2.57±0.17***
38-40	3.58±0.08***	2.18±0.10**	1.74±0.09
Control (nonpregnant women)	1.62±0.08	2.25±0.10	0.75±0.07

Note. *p<0.001, **p<0.01, ***p<0.05 compared to weeks 24-28, ^+p<0.001 compared to the control.

TABLE 2. Serum CP and TF Content (Arb. Units) in Pregnant Women with Gestosis of Different Severity ($M \pm m$)

Parameter	Normal pregnancy	Gestosis severity		
		mild	moderate	severe
CP	3.68±0.11	4.09±0.09***	2.71±0.13***++	2.50±0.23**++†
TF	2.04±0.09	1.82±0.09	2.64±0.14**+	2.78±0.14***+
CP/TF	1.80±0.06	2.47±0.19**	1.12±0.10*+	0.96±0.12*+

Note. *p<0.001, **p<0.01, ***p<0.05 compared to normal pregnancy; +p<0.001, ++p<0.01, +++p<0.05 compared to mild gestosis.

sation of the mechanisms regulating free radical processes occurred in moderate gestosis.

Hence, the study of CP and apo-TF concentrations is important. It is known that menstruation is accompanied by intensive loss of copper, and therefore, the increase in copper content during the first and second trimesters can be due to absence of menstruation. Later, copper reserves in maternal body are exhausted because of its active transfer through the placenta. CP content also decreases due to the same processes. Activation of hemopoiesis also contributes to decreased content of CP during the third trimester [11].

EPR spectroscopy with spin probes revealed a marked increase in EM microviscosity (1.47 times, p<0.01) and hydrophilia (1.38 times, p<0.01) of the phospholipid bilayer in severe gestosis. Moreover, the absolute potential of cell surface membrane increased by 20% and the lability of structural parameter of protein exposure on the membrane surface increased. The latter parameter reflects the severity of gestosis and helps to correct its therapy. In severe nephropathy, the content of aggregated proteins increases 1.53-fold (p<0.05) and the parameters of free lipid oxidation increase with the severity of gestosis [8]. All these changes in EM can lead to the formation of intermolecular lipid and lipid-protein cross-links, changes in the lipid matrix (increased microviscosity of the bilayer, cross-linking and inhibition of rotatory and lateral mobility of membrane proteins, facilitated phospholipid flip-flop, oligomerization and aggregation of membrane proteins, and alteration of the morphology of intramembrane lipid particles). Dysproteinemia at the expense of CP and TF, i.e. natural antioxidants, impaired the structure of EM proteins, disruption of disulfide bonds, exposure of SH groups, and reorganization of the protein-lipid relationships (when both integral and surface proteins and glycoproteins are involved in structural rearrangement) contribute to the realization of the studied phenomenon in gestosis [11].

The fluidity of the lipid bilayer depends on the mobility of carbon atoms in the phospholipid carbohydrate chain, concentration of bivalent cations, and cholesterol content. Loose packing of phospholipids with unsaturated and branched fatty acid residues also contributes to increased membrane fluidity. Ca and Mg ions decrease electrostatic repulsion of charged phospholipid heads, which promotes more compact packing of molecules in the bilayer, *i. e.* limits mobility of fatty acid chains and decreases membrane fluidity. Lipids participating in the organization of the lipid bilayer maintain the conformation status of membrane enzymes, regulate the interactions in oligomeric complexes, whereas the properties of lipid molecules depend on their location (at the lipid-protein interface or in protein-free areas) [1].

Hence, the severity of gestosis is characterized by increased microviscosity of both surface and deep membrane layers, impairment of protein-lipid interactions, activation of LPO against the background of decreased AOA and accumulation of aggregated material in EM, which characterizes the severity of LPO—AOA imbalance and determines to a certain extent the prognosis and type of complications of gestosis.

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