

EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Blood Antioxidant Defense during Early Terms of Pregnancy

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Threatened abortion at the early critical terms of gestation is characterized by drastic changes in the antioxidant status of the blood: activation of the pentose phosphate pathway of energy metabolism in erythrocytes and intensification of lipid peroxidation in erythrocytes and plasma.

Key Words: pregnancy; antioxidant defense of the blood; antioxidant enzymes

There are 3 gestation periods critical for fetal development: implantation, organogenesis, and placentation (the first 3 months of intrauterine period), the period from week 3 through 6 (organogenesis) being the most sensitive phase. Organogenesis is characterized by active differentiation of tissues; the fetus during this period is highly sensitive to internal and external damaging factors. Development of the vascular placenta is another critical period. Chronic placental insufficiency is observed in about 30% women at a high risk of spontaneous abortion. Lipid peroxidation (LPO) causes membrane insufficiency and abnormalities, including placental insufficiency [6,8]. In pathological pregnancy, *e. g.*, in gestosis, the disease severity correlates with LPO intensification [3]. However, the role of LPO during the early terms of gestation (placentation and early organogenesis, *i. e.* weeks 3-6 and 8-12, respectively) is little studied [7]. We investigated the antioxidant status of the blood during the early critical terms of pregnancy in patients at a high risk of miscarriage.

MATERIALS AND METHODS

Antioxidant defense of the blood was evaluated in 11 pregnant women at risk of spontaneous abortion with

clinical symptoms of threatened miscarriage (pain, bloody discharge), confirmed in some cases by paraclinical examinations: echography, evaluation of the cell immunity by latex agglutination test (LAT) detecting disorders in the bilateral immunomodulating mechanism of maternal immunity, α -fetoprotein assay. In parallel, antioxidant status of the blood was studied in 8 women with normal gestation and in 16 healthy nonpregnant women. The age of examinees was 19-35 years. The studies were carried out during critical periods of the first trimester: at weeks 3-6 and 8-12.

Erythrocytes were separated from plasma, washed 3 times, and glucose-6-phosphate dehydrogenase (G-6-PD), glutathione peroxidase (GP), and TBA-reactive products were measured [1,2,4]. Measurements of G-6-PD provide valuable information on the antioxidant status, because the hexose monophosphate pathway leads to the formation of NADPH utilized by glutathione reductase directly involved in the maintenance of reduced glutathione pool. Plasma GP activity was measured; chemiluminescence was evaluated on an EMILITE EL 1105 chemiluminometer (BioChemMac) [10]. Luminol-dependent chemiluminescence was studied using hydrogen peroxide as the inducer. The concentrations of hydroperoxides, plasma oxidation, and rate of induced oxidation were evaluated.

The results were processed by methods of variation statistics using Student's *t* test.

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TABLE 1. Antioxidant Status of Erythrocytes in Various Terms of Pregnancy ($M \pm m$)

Group	Term, weeks	G-6-PD, U/g Hb	GP, U/g Hb	TBA-reactive products, nmol/g Hb
Healthy women		76.8 \pm 1.4	36.3 \pm 2.0	123.4 \pm 3.2
Normal pregnancy	3-6	102 \pm 3*	32.5 \pm 1.9	141.9 \pm 35.1
	8-12	104.1 \pm 3.9*	31.2 \pm 1.6	153.6 \pm 44.5
Risk of miscarriage	3-6	91.1 \pm 2.3*	32.5 \pm 0.9	177.1 \pm 19.2*
	8-12	97.5 \pm 4.3*	33.4 \pm 1.3	166.8 \pm 18.0*

Note. Here and in Table 2: * $p < 0.05$ vs. healthy women.

RESULTS

The concentration of TBA-reactive products in erythrocytes tended to increase during pregnancy (Table 1). In patients at risk of spontaneous abortion, the concentration of TBA-reactive products considerably increased. Activity of antioxidant enzyme GP in erythrocytes was virtually the same in normal pregnancy and in pregnancy at risk of spontaneous abortion during both critical periods. Activity of G-6-PD at the early terms of gestation was appreciably increased indicating the involvement of compensatory reactions aimed at protection of erythrocytes from LPO. There were no notable differences between G-6-PD levels during the 3-6th and 8-12th weeks of gestation or between the levels of the enzyme in normal pregnancy and risk of miscarriage.

In the plasma, activity of antioxidant enzyme GP tended to pronounced decrease in women at risk of miscarriage at very early (3-6 weeks) terms (Table 2).

Investigation of LPO in the plasma by the chemiluminescent method showed very interesting results. All parameters characterizing plasma chemiluminescence were sharply increased in pregnant women in comparison with nonpregnant young women. In normal pregnancy, the concentration of hydroperoxides increased 4.2- and 4.5-fold at weeks 3-6 and 8-12, respectively. The highest concentration of hydroperoxides in the plasma was detected in patients at high

risk of spontaneous abortion during the 8-12th weeks: plasma oxidation 4.6-fold surpassed the normal.

The rate of induced LPO in the plasma increased approximately 3-fold both in normal pregnancy and in patients at risk of miscarriage (differences are insignificant).

The drastic increase in the level of plasma chemiluminescence indicates impaired antioxidant capacity of the blood in pregnant women. Some authors believe that fetal hypoxia can be detected by chemiluminescent methods because LPO is intensified in hypoxia [5]. Hence, the severity of hypoxia directly correlates with the content of LPO products in the blood. LPO intensification together with other factors, plays an important role in the development of gestoses, and the concentration of LPO products correlates with the severity of pregnancy complications [9,11]. All this indicates that insufficiency of the blood antioxidant system may promote disturbances in the mother-fetus system. For the first time we detected inadequate antioxidant system of the blood during very early critical periods of normal gestation and in patients at risk of miscarriage.

Therefore, the early critical periods of pregnancy are characterized by drastic changes in the antioxidant capacity of the blood:

- ♦ activation of the pentose phosphate pathway of energy metabolism and intensification of LPO in erythrocytes, confirmed by increased concentrations of TBA-reactive products;

TABLE 2. Antioxidant Status of the Plasma in Various Terms of Pregnancy ($M \pm m$)

Group	Term, weeks	GP, U/liter	Plasma chemiluminescence, arb. units		
			hydroperoxide concentration	plasma oxidation	rate of induced LPO
Healthy women		641.3 \pm 11.2	21.9 \pm 3.3	125.8 \pm 4.3	3.93 \pm 1.58
Normal pregnancy	3-6	582.9 \pm 30.2	92.0 \pm 17.6*	454.0 \pm 60.1*	11.7 \pm 1.5*
	8-12	521.0 \pm 46.5	99.5 \pm 18.9*	464.0 \pm 78.3	12.2 \pm 2.0*
Risk of miscarriage	3-6	460.2 \pm 64.1	67.8 \pm 20.3	354.2 \pm 100.0*	7.2 \pm 1.6*
	8-12	497.3 \pm 35.0	120.9 \pm 35.0*	577.2 \pm 158.0*	10.9 \pm 1.9*

- ◆ intensification of LPO in the plasma confirmed by increased chemiluminescence and suppressed GP activity;
- ◆ inhibition of GP in the plasma and accumulation of TBA-reactive products in erythrocytes more pronounced in women at risk of spontaneous abortion.

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