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LABORATORY CRITERIA OF PERINATAL DAMAGE OF CENTRAL NERVOUS SYSTEM AT PREMATURE NEWBORNS

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ABSTRACT
Introduction: Fetal and neonatal hypoxia takes a special place among the damaging factors of central nervous system (CNS). All forms of oxygen deficiency are accompanied by the development of bioenergetic hypoxia, which leads to tension of metabolic processes of the organism. Metabolic effect of hypoxia includes stark reduce of mitochondrial activity due to a significant inhibition enzymes of the Krebs cycle: succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH). In newborn babies is not always possible to objectively assess the condition of the CNS defeat, because very often the severity of lesions does not correspond to clinical symptoms, especially in premature newborns. So far determination the severity of hypoxic-ischemic CNS lesions is still very actual in modern medicine. More objective method of such an assessment is determine the activity of neurospecific enolase (NSE).

The aim of the paper is to increase the efficiency of diagnosis of hypoxic CNS lesions in premature infants by determining the activity of NSE and study energy supply during the neonatal period.

Materials and methods: The concentration of NSE, SDH and LDH were determined in 15 conventionally healthy preterm infants (CHPI), which made the comparison group, and 64 premature babies with hypoxic-ischemic CNS lesions, which were divided into three groups: I group - 26 premature children with mild CNS lesions; II group - 20 premature children with severe hypoxic lesions and low birth weight; III group - 18 premature newborns with severe damage of central nervous system and extremely low birth weight. NSE activity was determined by enzyme immunoassay using reagents of the company «Fujirebio» (Sweden) on an automatic analyzer «Multiscan Plus» company «Labsystems» (Finland). Material for investigation was peripheral venous blood of newborns, which collected by vein punction at morning on an empty stomach.

Results and conclusions: Metabolic effect of hypoxia in premature infants manifested by severe inhibition of mitochondrial respiratory activity, which appears in the reduction of aerobic enzyme activity of SDH and activation serum LDH. During the neonatal period in infants with perinatal hypoxic-ischemic lesions of the CNS levels of the NSE aren't normalized, that indicated on energy deficiency and requires the development of effective methods of correcting this condition. Perinatal hypoxia in premature neonates causes significant alteration of neuronal membranes and increase concentration in blood such neurospecific protein as NSE, whose concentration correlates with the degree of severity of CNS injury.

KEY WORDS: hypoxia, succinate dehydrogenase, lactate dehydrogenase, neurospecific enolase, premature newborns

INTRODUCTION
Fetal and neonatal hypoxia takes a special place among the damaging factors of central nervous system (CNS). This pathology usually is a result of placental insufficiency, which oversees almost all complications of pregnancy - toxicsis, intrauterine growth retardation, prematurity, infection. More important place hypoxic damage occurs in premature infants, in which it is 10-15 times more often cause death of children [1].

All forms of oxygen deficiency are accompanied by the development of bioenergetic hypoxia, which leads to tension of metabolic processes of the organism [2]. Metabolic effect of hypoxia includes stark reduce of mitochondrial activity due to a significant inhibition enzymes of the Krebs cycle: succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH).

Some authors believe that activity of oxidative enzymes of lymphocytes, particularly succinate dehydrogenase, is adequate reflection of dysmetabolical processes and energy cell metabolism [3, 4].

Abovementioned enzymes (SDH, LDH) are key in the processes of aerobic and anaerobic glycolysis, and decrease their activity - is as a marker of whole mitochondrial dysfunction [5, 6]. Therefore, objective criteria for assessing the severity of CNS lesions are indicators of cells energy metabolism.

Mechanisms of hypoxic damage of brain cells are characterized by a complex cascade of pathophysiological processes. Final result of this mechanism is the death of neurons due to necrosis and apoptosis [7].

In newborn babies is not always possible to objectively assess the condition of the CNS defeat, because very often the severity of lesions does not correspond to clinical symptoms, especially in premature newborns. So far determination the severity of hypoxic-ischemic CNS lesions is still very actual in modern medicine. More objective method of such an assessment is determine the activity of neurospecific enolase (NSE) [7].
THE AIM
The aim of the paper is to increase the efficiency of diagnosis of hypoxic CNS lesions in premature infants by determining the activity of NSE and study energy supply during the neonatal period.

The objects of research are premature infants with hypoxic CNS lesions of different severity.

MATERIALS AND METHODS
The concentration of NSE, SDH and LDH were determined in 15 conventionally healthy preterm infants (CHPI), which made the comparison group, and 64 premature babies with hypoxic-ischemic CNS lesions, which were divided into three groups:
- I group – 26 premature children with mild CNS lesions;
- II group – 20 premature children with severe hypoxic lesions and low birth weight;
- III group – 18 premature newborns with severe damage of central nervous system and extremely low birth weight.

Severity of hypoxia was determined by considering pregnancy and childbirth, state after birth (Apgar score, signs of dysfunction of the central nervous system, respiratory, cardiovascular and urinary systems in the first three days of life), laboratory (concentration of urea and creatinine in blood, urine analysis, blood pH) and instrumental (ultrasound) data.

NSE activity was determined by enzyme immunoassay using reagents of the company «Fujirebio» (Sweden) on an automatic analyzer «Multiscan Plus» company «Labsystems» (Finland). Material for investigation was peripheral venous blood of newborns, which collected by vein punctation at morning on an empty stomach.

The energy supply of the newborns was evaluated after activity of succinate dehydrogenase (SDH) in blood lymphocytes and lactate dehydrogenase (LDH) in serum of blood.

SDH activity in the lymphocytes of peripheral blood determined by quantitative cytchemical method using reagents of the company «SIGMA-ALDRICH» (Suisse).

Anaerobic metabolism in newborns was studied through measuring LDH activity in plasma by kinetic method on semi-automatic photometer PV 1251(Byelorussia) using reagent kits of the company “Diacom” (Russia) according to a standardized method that optimized by German Society for Clinical Chemistry – test pyruvate – lactate.

The content of enzymes in the blood was studied in the early neonatal (1-7th days of life) and late neonatal (20-30th days of life) periods.

Statistical analysis of results carried out with a program Microsoft Excel. We used statistics variation methods which are suitable for medical and biological research. For all parameters measured the average (M), the average error (m). Using the Student's criterion (t) determined reliability index (R).

RESULTS AND DISCUSSION
The average gestational age and body weight of babies in the different groups were respectively: 33,77 ± 0,26 weeks and 2024 ± 32,1 g in the first group, 33,16 ± 0,58 weeks and 2015,48 ± 34,2 g – in the second, and 28,88 ± 0,72 weeks and 1126,48 ± 24,3 g – in the third group. The comparison group included children which were born at term 35,26 ± 0,51 weeks, body weight was 2194,8 ± 81,11 g.

Determining the level of NSE in serum of premature infants found that at the end of the early neonatal period in brain cells of children with hypoxic-ischemic CNS lesions showed destructive changes of neuronal membranes. About this evidenced significant increase the level of enzyme. So, if perinatal CNS lesions of mild degree occur, NSE content in the blood of children of I group increased by 45% relative to the comparison group (p <0,05). Thus, even mild hypoxia caused a significant alteration of neuronal membranes and damage brain tissue. In the second group of infants with low birth weight on the base of severe hypoxia there was further increase activity of this enzyme in the blood, which manifested by increased serum concentrations of NSE in 2,2 times relative to the children of I group (p <0,001). It should also be noted that its activity in case of severe hypoxia was almost 3,3 times higher relative to the comparison group (p <0,001).

Maximum concentration of enolase reached in premature infants with very low birth weight and severe hypoxic-ischemic injury of the CNS. Its contents in serum of premature neonates of III group was 4 times greater than in comparison group (p <0,001), increased 2,9 (p <0,001), and 1,3 (p <0,05) times relative to infants of I and II groups, respectively (Figure 1).

Thus, hypoxic injury of the nervous tissue causes increased permeability of cell membranes and leave into the blood such neurospecific protein as NSE. The high rates of NSE in serum of premature infants on a base of hypoxic injury describe breach of the functional condition of cell membranes of neurons and correspond to the severity of brain damage due to hypoxia [7]. Therefore, to assess the severity of hypoxia is necessary determine the level of NSE in serum in the early neonatal period in premature infants.

During the neonatal period in the serum of all groups of children one can see a significantly lower enolase concentration, indicating a gradual recovery of neurons. It should be noted that at the end of the 30th day of life the level of this enzyme in premature infants with hypoxic-ischemic CNS lesions remained significantly higher than in comparison group. In the children of I, II and III group it was 1,4, 2,8 and 3,5 times greater than in the comparison group of kids.

So, in premature infants in the case of perinatal hypoxic-ischemic CNS lesions in the absence of any clinical symptoms at the end of neonatal period there is no stabilization of cell membranes of neurons, as indicated by high rates of neurospecific enolase in serum. These data suggest the possibility of remote effects due to central nervous system injury and the need to control the level of NSE during the neonatal period [8,9].

Furthermore, perinatal hypoxia may cause tension all metabolic processes in the organism. Changes of energy
metabolism can manifest by violation of almost all organs and systems, especially the central nervous system, heart and kidneys. Most accessible cells, which indicates early metabolic shifts in the body, are peripheral blood lymphocytes. Enzyme activity of lymphocytes is a "enzymatic mirror" of metabolic processes in different tissues. An oxidative enzyme succinate dehydrogenase is a marker of energetic processes of Krebs cycle. This enzyme is a part of the complex II of mitochondrial respiratory chain. It is strongly associated with the inner membrane of mitochondria, and the low activity of SDH evidences about inhibition of Krebs cycle functions. Enzymatic status of lymphocytes provides an opportunity to assess the degree of hypoxic influence on the newborn organism [10, 11].

In the premature newborn metabolic effect of hypoxia occurs in the early neonatal period through severe inhibition of mitochondrial respiratory activity, as testified by reduced activity of the main aerobic enzyme - SDH.

In infants with mild perinatal CNS injury in the early neonatal period the total number of granule of formazan into cells decreased on 33% relative to the comparison group ($p < 0.001$). In same time for infants in second and third groups were quantity 1.7 and 2.0 times lower respectively than in comparison group ($p < 0.001$) (Table I).

Along with a decreased of the total number of formazan granules, were decreased number of cells containing these granules, and the average number of granules per cell.

Reduced activity of SDH indicates the initial stages of the process of decompensation, which is accompanied by disturbance of energy metabolism and leads to the formation of hypoxia. At severe CNS injury further decrease activity of this enzyme shows the progression of decompensation and the formation of severe energetical disorders.

During the neonatal period in peripheral blood lymphocytes in premature newborns with hypoxic-ischemic CNS lesions observed only a trend towards recovery SDH activity. Even at the end of the 30th day, the total number of formazan granules in infants with severe hypoxia was 1.5 times less relative to the comparison group. Significantly low remained also the number of cells containing the enzyme.

These data indicate the ineffectiveness of aerobic glycolysis even at the end of the first month of life, so you can talk about development in premature infants energetical deficiency due to hypoxic and ischemic CNS lesions and requires the development of effective methods of correction.

Thus, the results of cytochemical studies of lymphocytes found that in premature infants with perinatal hypoxic-ischemic CNS lesions occurred pronounced changes in metabolic adaptation [12,13].

The brain, as the main target organ in case of hypoxia, is very sensitive to hypoxia. Energy supply of the brain caused most of all by aerobic mechanisms. Hypoxia causes an energy stress that activates compensatory anaerobic way of glucose utilization, for which is high specific increase activity of anaerobic enzymes, especially LDH.

Research LDH levels in serum of premature infants with perinatal hypoxic-ischemic CNS lesions found that in case of oxygen deficiency in children of all groups was significant increase level of this enzyme, and thus activation of anaerobic glycolysis. In the early neonatal period in children with mild hypoxic lesions enzyme concentration increased 2.5 times relative to the comparison group ($p < 0.001$). This shows the maximum tension of compensatory adaptive mechanisms aimed to the effective utilisation of energy substrates to prevent the energy deficiency.

In case of severe hypoxia observed certain exhaustion of compensatory mechanisms of anaerobic glycolysis activation, but even among newborns of second and third groups with severe hypoxic injury LDH level in serum was 1.7 times greater ($p < 0.001$ and $p < 0.01$, respectively) than in neonatal comparison group (Figure 2).

During the neonatal period, the gradual recovery of aerobic glucose utilization way was found. Indicator of this process was some reduction of serum LDH in the newborns with perinatal hypoxic lesions of the CNS. Significant
Table 1. Morphocytochemical indicators of SDH activity in peripheral blood lymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>Total number of granules</th>
<th>Total number of cells with granules</th>
<th>Number of granules per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 day</td>
<td>424,62±14,9</td>
<td>43,5±0,5</td>
<td>9,76±0,3</td>
</tr>
<tr>
<td>20-30 day</td>
<td>417,43±19,2</td>
<td>43,4±0,5</td>
<td>9,66±0,5</td>
</tr>
<tr>
<td><strong>I group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 day</td>
<td>319,0±17,3</td>
<td>36,4±0,7</td>
<td>8,73±0,4</td>
</tr>
<tr>
<td>20-30 day</td>
<td>358,06±20,1</td>
<td>39,9±0,7</td>
<td>8,95±0,4</td>
</tr>
<tr>
<td><strong>II group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 day</td>
<td>249,37±10,4</td>
<td>32,5±0,9</td>
<td>7,70±0,4</td>
</tr>
<tr>
<td>20-30 day</td>
<td>260,75±11,6</td>
<td>34,2±0,9</td>
<td>7,74±0,4</td>
</tr>
<tr>
<td><strong>III group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 day</td>
<td>207,87±18,1</td>
<td>30,6±1,9</td>
<td>6,72±0,2</td>
</tr>
<tr>
<td>20-30 day</td>
<td>233,5±12,6</td>
<td>32,8±1,1</td>
<td>7,09±0,2</td>
</tr>
</tbody>
</table>

Notes: p – significantly different of indexes relative to the comparison group; p₁ – significantly different of indexes relative to the 1st group; p₂ – significantly different of indexes relative to the 2nd group.

Fig. 2. Dynamics of LDH in premature babies with hypoxic-ischemic injury of the central nervous system, mg/ml.

decrease concentration of this enzyme was observed only in children with mild hypoxia. But even at the end of the first month LDH contents in the blood of children of all groups with hypoxia remained significantly higher than in comparison group, that indicated the absence of adequate recovery of energy metabolism into cells.

Thus, the process of aerobic glycolysis occurs in the mitochondria of brain cells with enzymes of Krebs cycle, the main of which is SDH, only in case of normal diffusion oxygen from intercellular space into neurons [14,15].

Hypoxia dramatically reduces the activity of aerobic glycolysis, which is manifested by low activity of such enzyme of Krebs cycle as SDH. Metabolism in brain cells become anaerobic with the activation of the corresponding enzyme (LDH) [16, 17]. Functional brain activity is suppressed, which is manifested clinically by progressive impairment of consciousness. Hypoxia blocks include pyruvic acid into Krebs cycle, that's why piruvate isn't oxidized and converted to lactic acid. Increasing the concentration of the latter causes acidosis, which is a factor of destruction of cell membranes of neurons. Violation of the integrity of neuronal membranes leads to increase concentration in blood neurospecific marker of brain cells damage – NSE, whose concentration in serum of newborns with hypoxic CNS lesions increases dramatically [18, 19].

Thus, as markers of severity of perinatal hypoxic-ischemic CNS lesions in premature infants during the neonatal period can use activity of such indicators as SDH, LDH and NSE.
CONCLUSIONS

1. As markers of severity of perinatal hypoxic-ischemic CNS lesions in premature infants during the neonatal period can use activity of such indicators as SDH, LDH and NSE.

2. Metabolic effect of hypoxia in premature infants manifested by severe inhibition of mitochondrial respiratory activity, which appears in the reduction of aerobic enzyme activity of SDH and activation serum LDH. During the neonatal period in infants with perinatal hypoxic-ischemic lesions of the CNS levels of the of NSE, SDH and LDG aren’t normalized, that indicated on energy deficiency and requires the development of effective methods of correcting this condition.

3. Perinatal hypoxia in premature neonates causes significant alteration of neuronal membranes and increase concentration in blood such neurospecific protein as NSE, whose concentration correlates with the degree of severity of CNS injury.

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Conflict of interest:
The Authors declare no conflict of interest.