Comparison of S100 protein and MDA concentrations in blood of newborns and infants with hypoxic-ischemic encephalopathy demonstrated difference in the parameters by a type of cerebral ischemic syndrome. Follow-up study of serum S100 protein concentrations demonstrated absence of normalization in the parameter in patients with psychomotor retardation and paroxysmal syndrome, no normalization of MDA level found in examinees with emotional and behavioral disorders and psychomotor retardation.

**Keywords:** Central nervous system (CNS), hypoxic-ischemic injuries, membrane destruction

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**Introduction**

Impairment in balance between intensity of generation of oxygen active forms and condition of anti-oxidant system induces various diseases (Soatov, Ibragimov, and Khaibullina, 2009). Hypoxic-ischemic central nervous system (CNS) injuries can be identified as one of pressing problems of today pediatric neurology due to complications resulting in neuropsychic dysfunctions in children. Cerebral hypoxic-ischemic injuries constitute a significant part of CNS diseases in infants (Bulakhova and Belikova, 2007; Volodin, Medvedev, and Rogatkin, 2001; Shamansurov, Sokhieva, and Uzakova, 2010). Cerebral tissues are the main consumers of total oxygen supplied to a human’s organism, insignificant reduction in oxygen partial pressure results in cerebral ischemia development. Some authors reported that CNS ischemic injuries enhance membrane destructive processes in neurocytes and glial cells resulting in release of their specific components into circulation. Appearance of neuropeptides and neuron specific enolase (NSE) in the circulation can be an indication of membrane destruction (Khalimbetov, 2011; Barashneva, 2001). However, interrelation between the membrane destruction and enhancement in release of neuropeptides into circulation is still unclear. The work was initiated to study interrelation between manifestations of membrane destruction and circulating concentrations of neuropeptides upon CNS hypoxic-ischemic injuries in newborns and infants.

**Materials and methods**

We examined 110 patients with CNS hypoxic-ischemic injuries aged from 3 to 14 months hospitalized at the pediatric neurology department of Tashkent City Children’s Hospital No.1. The examinees were divided into four groups. Thus, 30 patients with psychomotor retardation, 23 subjects with a psycho-speech disorder, 28 children with emotional and behavioral disorders (EBD) and 29 examinees with paroxysmal syndrome were included into the groups. 20 children without perinatal CNS injury and infectious inflammatory diseases comprised the control group. Neurosonography was performed during physiological sleep. The patients were examined within first three months of life and in 14 months following the rehabilitation period.

EIA, a solid-phase, non-competitive immunoassay with commercially available kits (CanAg, Sweden) was used to measure serum neuropeptides at admission and in 12-14 months. The standards and samples were incubated with biotinylated anti-S100
monoclonal murine antibodies in streptavidin-covered microplate cells. Microplate reader was used to measure color intensity at 405 nm following addition of a stop solution. Serum malondialdehyde concentration was measured by a method described elsewhere (Nagoev and Tulupova, 2008) to be recalculated for the total protein content.

Results and discussion

Close correlation between manifestations of CNS perinatal injury in infants and character of hypoxic effect was previously demonstrated. Thus, the author showed that perinatal hypoxia in newborns facilitates disturbance of both function and structure of cell membranes to be associated with excessive activity of lipid peroxidation processes, accumulation of diene conjugates and Shiff bases with underlying low activity of antioxidant protective enzymes (Khalimbetov, 2011). In the cells of various organs and systems intensity of generation of oxygen active forms is in certain balance with the antioxidant system condition (Soatov et al., 2009). Disturbance of the balance is the factor facilitating membrane destruction and release of intracellular components into peripheral circulation.

At admission significant increment in malondialdehyde (MDA) was registered in blood of the patients (Table). Serum MDA was the highest in peripheral circulation of examinees with EBD, exceeding the control values by 3.65 times. MDA concentrations in peripheral circulation of infants with paroxysmal syndrome were higher than in subjects comprising control group by 3.25 times (Table). MDA increase was found the lowest in patients with psychomotor retardation exceeding the control values by 2.08 times (P<0.05). In 14 months serum MDA concentrations were found significantly reducing in infants with psycho-speech disorders and in those with paroxysmal syndrome, not differing from the values in the control group. MDA increase was found the lowest in patients with psychomotor retardation and EBD were higher than those in the control group by 1.49 and 1.61 times, respectively. MDA is formed resulting from interaction of oxygen active forms with macromolecules of lipid nature, such as, cell membrane phospholipids and polyunsaturated fatty acids being the part of membrane phospholipids. Upon membrane destruction membrane permeability as well as release of components of nerve cells into circulation is found increasing. In blood of newborns with hypoxic-ischemic syndromes increase in S100 protein concentrations were found exceeding the control values by 5.2 and 3.7 times (Table). Concentrations of neuropeptides were the highest in newborns and infants with paroxysmal syndrome, lower release of neuropeptides registered in examinees with EBD. This difference can be attributed to more serious injury of synapsis observed upon paroxysmal syndrome than upon EBD. The data for S-100 protein in the Table is the statistically average not allowing comparison with the data of clinical picture manifestation. High serum S100 protein concentrations were registered in 6 of 29 (20.7%) newborns and infants with paroxysmal syndrome. Contraction in dorsal, femoral and brachial muscles, enhanced muscular tension on the right leg as well as shuddering in the sleep were typical for the patients of this group. Analysis of their mothers’ medical histories revealed eclampsia and anemia. But in 10 patients (34.5%) with paroxysmal syndrome peripheral S100 protein and MDA concentrations were lower than the statistically average values. Thus, if MDA release is a criterion for membrane destruction lower S100 protein concentrations are the indirect confirmation of the fact. Clinically lower frequency of contractions in musculus gastocnemius as well as in elbow joint flexors and extensors was observed in patients of this group as compared with the patients in the previous group. The contractions were tonic and impermanent to be easily arrested. The lowest release of S100 protein into peripheral circulation was found in patients with EBD; though in 6 (21.4%) lower concentrations of the latter in relation to the statistically average values were registered. Consequently, in this case there were lower serum MDA concentrations. However, 7 patients (25%) had higher serum S100 protein concentrations to be associated with behavioral disorders, such as, weeping without cause, anxiety, nursing strike. In these examinees MDA concentrations were found higher as well. The
findings are consistent with those reported by other authors (Volodin et al., 2001; Golosnaya, Petruchkin et al., 2004). Perinatal hypoxia is believed to initiate processes facilitating elevation of cell membrane permeability, death of neurons and glial cells due to necrosis or apoptosis, disturbance of blood-brain barrier integrity and release of cerebral antigens into circulation. S100 protein is found to be the most sensitive to changes in calcium homeostasis promptly responding to the acute cerebral ischemia. Its appearance in the blood serum can be the evidence for destruction of neuronal membranes, calcium intracellular accumulation and subsequent responses of glutamate-calcium cascade. Our findings demonstrated that release of S100 protein into blood serum depends not only on perinatal pathology severity, but also on the type of neurological syndrome. Follow-up study performed 14 months later demonstrated the lowest serum S100 protein concentrations in patients with EBD slightly exceeding the parameter in the control group (P>0.05). Serum S100 protein concentrations in other groups of newborns and infants remained higher than those in the control group subjects as well (P<0.05).

In the group of patients with psychomotor retardation 4.8-time higher increase in the serum S100 concentrations as compared with the one in the control group newborns and infants was found, while in examinees with psycho-speech disorder they were 4.2 times higher than those in the control group.

Thus, S100 protein release into circulation associated with membrane destruction process determined by serum MDA accumulation was found varying in groups of newborns and infants with various types of syndromes. Determination of S100 protein and MDA concentrations in peripheral circulation allowed differentiating post-hypoxic clinical syndromes and assessing number of affected cerebral cortex neurocytes.

Follow-up findings demonstrated absence of normalization in serum S100 protein concentrations in patients with psychomotor retardation and paroxysmal syndrome and in MDA levels in patients with EBD and psychomotor retardation to constitute the basis for development of appropriate pathogenetic therapy for patients with the nosology.

Some authors report on more frequent psycho-speech disorders, nervous system pathology and changes in psychological status in children with mild and moderate perinatal nervous system injury (Golosnaya et al., 2004; Yaremenko, Yaremenko, and Goryainova, 2002). Neuron specific proteins are widely used in diagnosis of various CNS pathological changes (Pekny, Jonasson et al., 1999). The findings can serve as the evidence for interrelation between intensity of membrane destructive process (serum MDA accumulation) and S100 protein release into circulation, the intensity varying by the type of cerebral ischemic syndrome.

**TABLE 1. Concentrations of MDA and S100 Protein in Blood of Newborns and Infants with Various Syndromes of CNS Hypoxic-Ischemic Pathology**

<table>
<thead>
<tr>
<th>Type of syndrome</th>
<th>Basal MDA (µmol/l)</th>
<th>Basal S100 protein (mkg/l)</th>
<th>In 14 months MDA (µmol/l)</th>
<th>S100 protein (mkg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychomotor retardation (n=30)</td>
<td>1.06 ± 0.15</td>
<td>364.5 ± 13.4</td>
<td>0.76 ± 0.07</td>
<td>142.5 ± 7.2</td>
</tr>
<tr>
<td>Psycho-speech disorder (n=23)</td>
<td>1.24 ± 0.11</td>
<td>320.2 ± 10.1</td>
<td>0.65 ± 0.08</td>
<td>87.9 ± 4.9</td>
</tr>
<tr>
<td>EBD (n=28)</td>
<td>1.86 ± 0.25</td>
<td>278.5 ± 13.1</td>
<td>0.82 ± 0.06</td>
<td>92.8 ± 4.8</td>
</tr>
<tr>
<td>Paroxysmal syndrome (n=29)</td>
<td>1.66 ± 0.25</td>
<td>406.4 ± 13.4</td>
<td>0.62 ± 0.06</td>
<td>128.1 ± 9.2</td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>0.51 ± 0.08</td>
<td>76.1 ± 3.2</td>
<td>0.51 ± 0.08</td>
<td>76.1 ± 3.2</td>
</tr>
</tbody>
</table>
Conclusion

Comparison of S100 protein and MDA concentrations in blood of newborns and infants with hypoxic-ischemic encephalopathy demonstrated difference in the parameters by a type of cerebral ischemic syndrome. Follow-up study of serum S100 protein concentrations demonstrated absence of normalization in the parameter in patients with psychomotor retardation and paroxysmal syndrome, no normalization of MDA level found in examinees with EBD and psychomotor retardation.

References