Blood Immunological Parameters upon Hypoxic-Ischemic Injuries of Central Nervous System in Newborns and Infants

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Introduction

Perinatal hypoxic-ischemic central nervous system (CNS) injuries in newborns and infants as well as their complications are the urgent problems of modern pediatric neurology. Barashneva (2001) and Volodin (2007) report that complications of cerebral tissue hypoxia in newborns with brain ischemia have high pathogenetic significance. Other authors highlight the significance of brain hypoxia in perinatal pathology as well (Zykov et al., 2003). Hypoxic affection of cerebral tissue has been assessed in the work reporting findings of CNS studies among infants with consequences of perinatal nervous system injuries (Shamansurov et al., 2010).

CNS ischemic injuries are associated with a complex of immunological and biochemical responses in nerve tissue. Some authors reported that CNS ischemic injuries enhance membrane destructive processes in neurocytes and glial cells resulting in release of S100 protein and neuron-specific enolase (NSE) into circulation. Interrelation between the release of neuropeptides and NSE into circulation and content of interleukins is still unclear. The work was initiated to study interrelation between immune system condition and circulating concentrations of neuropeptides and NSE upon perinatal pathology 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.

Content of interleukin-1 (IL1) and tumor necrosis factor-α (TNF-α) was studied in the culture of the patients’ blood mononuclear cells obtained from fresh heparinized blood culture by centrifugation in the renografin density gradient. 5x10^6/ml of cells were suspended in the enriched culture medium with 5% heat inactivated calf serum and incubated at 37°C for 1 hour. Nonadherent cells were washed out from culture plate, the rest being subcultured in the enriched medium with addition of 10 mg/ml of lipopolysaccharide for 6 hours. The supernatant was used to assay content of TNF-α and IL-1. Statistical processing was performed by means of data-processing math software packets.

Results and discussion

Membrane destructive process has been previously demonstrated to be associated with alterations in oxidant status power and free radical oxidation rate, changes in superoxide dismutase activity being the focus of many studies (Gorbenko, 2006; Korenovskyi et al., 2006). Enhancement of oxidative stress is known to result in membrane destruction and release of cytoplasmic components into blood. Content of circulating neuropeptides seems to be explained by the phenomenon. Membrane destruction is accompanied by the release of low-molecular products resulting from macromolecular degradation of lipid and protein molecules (Ibragimov et al., 2010) affecting receptors of immunocompetent cells and enhancing synthesis of interleukins and cytokines in the examinees with CNS hypoxic-ischemic pathology. Significant increment in peripheral circulating IL-1 was registered in the patients at the hospitalization.

The highest circulating IL-1 concentration exceeding the control level by 7.94 times was registered in infants with psychomotor retardation, similar increase being found in the group of patients with the paroxysmal syndrome. Increase of IL-1 was found parallel to high levels of S100 protein in 3 patients (10%) with psychomotor retardation. This is the case of membrane destruction with enhancement of synthesis and/or release of interleukins into circulation. However, there were two cases (6.7%) when rapid increase in S100 protein paralleled relatively low circulating IL-1. In 13.3% of the patients rapidly increased NSE was found simultaneous with IL-1 growth. In 4 patients (13.3%) less increase in IL-1 coincided with NSE insignificant activity. The findings may confirm the concept hypothesizing role of low molecular peptides of glial structure and NSE in activation of interleukin synthesis.

Analysis of circulating IL-1 growth in patients with paroxysmal syndrome demonstrated unidirectional character of changes with accumulation of circulating S100 protein. Higher IL-1 versus mean level was found in 7 patients (20.7%), lower than average level of IL-1 being found in 9 (31.4%). IL-1 increase coincided with high S100 concentrations in 2 patients of the group only, while lower level of IL-1 never paralleled low S100 protein content. Only in 2 patients low mean activity of NSE paralleled similarly low mean level of IL-1.

In patients with perinatal CNS injury accompanied with EBD IL-1 level was registered higher than in those in the control group by 7.6 times. It should be noted that in this group IL-1 ranging from 9.4-18.6 pg/ml was higher than the mean one in 35.7%. In 6 patients (21.6%) IL-1 was found lower than the mean value. In 2 patients of the group (7.1%) low IL-1 coincided with low level of circulating S100 protein. Similar coincidence of changes in IL-1 level in association with circulating NSE could be seen.

In patients with CNS hypoxic-ischemic injury accompanied by a psycho-speech disorder as compared with those in the control group IL-1 content was found increased by 6.4 times, being less than in other groups of patients (P>0.05). In this group of patients
circulating IL-1 increase was 4.1-5.4 times higher than the one in patients in the control group in 47.8%, the parameter being higher than the control one by 7.8-10.1 times in 30.4%. In 3 patients (13.0%) high concentrations of IL-1 were found to coincide with significant increase in S100 protein, the similar number of patients demonstrating considerable growth of circulating NSE activity.

Oxidizing effect of active oxygen forms on lipid membrane frame of neurocytes, glial cells, synapses and myelin sheath of nerve fibers underlies progression of CNS hypoxic-ischemic disorders (Khaibullina et al., 2010). The role of a cytokine influencing lipid metabolism (Bradley, 2008) upon this pathology is quite intriguing. TNF-α is an extracellular protein synthesized mainly by monocytes and macrophages, its amount increasing in various diseases, after hypoxias in particular. In our examinees with CNS hypoxic-ischemic pathology TNF-α was found increased by 3.4-4.1 times as compared with those in the control group (Table 1). The increase was found most pronounced in patients with perinatal CNS pathology accompanied by EBD, being less marked in those with psychomotor retardation.

<table>
<thead>
<tr>
<th>Type of syndrome</th>
<th>Basal α-TNF (pg/ml)</th>
<th>Basal IL-1 (pg/ml)</th>
<th>In 14 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-TNF (pg/ml)</td>
<td>IL-1 (pg/ml)</td>
<td></td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>27.2 ± 3.0</td>
<td>12.7 ± 1.0</td>
<td>15.3 ± 0.6</td>
</tr>
<tr>
<td>Psycho-speech disorder</td>
<td>29.1 ± 0.1</td>
<td>10.2 ± 0.2</td>
<td>12.1 ± 0.5</td>
</tr>
<tr>
<td>EBD</td>
<td>32.9 ± 3.0</td>
<td>12.2 ± 0.1</td>
<td>11.1 ± 0.7</td>
</tr>
<tr>
<td>Paroxysmal syndrome</td>
<td>31.8 ± 3.0</td>
<td>12.4 ± 0.1</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>8.0 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>8.0 ± 0.2</td>
</tr>
</tbody>
</table>

In 8 patients with CNS hypoxic-ischemic injury associated with EBD (28.6%) TNF-α concentrations were found increased by 2.8-3.7 times, the parameter being higher by 4.4-4.9 times as compared with the control values in 9 (32.1%). In patients with paroxysmal syndrome TNF-α levels were found exceeding the control value by 3.1-3.6 times in 7 patients (24.1%), being higher than the control value by 4.4-4.9 times in the similar number of patients of the kind.

As to findings in the group of patients with a psycho-speech disorder, they demonstrated 3.64-time increase in circulating TNF-α as compared with those in the control group. Degree of increase varied to be 2.7-3.4 and 4.0-4.6 times higher than the control values in 8 (34.8%) and 7 patients (21.7%), respectively. Increase of the cytokine concentrations in blood of the examinees was unidirectional parallel to changes in circulating S100 protein in 3 patients (13.0%) and those in circulating NSE increase in 4 (17.4%).

In patients with CNS hypoxic-ischemic pathology accompanied by psychomotor retardation circulating TNF-α growth was higher by 3.4 times as compared with the control value. In 9 of them (30%) cytokine concentrations were found increased by 2.3-3.2 times versus the control ones, the parameter being increased by 3.9-4.9 times as compared with the control values in 8 (26.7%). Unidirectional changes in TNF-α and S100 protein concentrations were observed in 3 patients of the group only (10%), synchronicity of alterations with the increase in NSE enzymia being registered in 9 (30.0%).

Considering CNS hypoxic-ischemic pathology as a defect of antioxidant system including super oxide dismutase it should be noted that according to Cunningham et al., (2005) the reduction of antiradical protective activity of the enzyme causes release of matrix metalloproteinase-9 from nervous tissue astrocytes. It is a well-known fact that TNF-α is synthesized as a membrane protein (Old, 1985; Bidwell et al., 1999). Under the effect of specific metalloproteinase a membrane-associated fragment is detached to form soluble TNF-α (Verstrepen et al., 2008).
Thus, a specific pathogenetic link including increase of cytokines and interleukins upon membrane destructive process caused by CNS hypoxic-ischemic pathology with release of neuropeptides and NSE into the circulation could be seen.

Retrospective analysis of TNF-α and IL-1 concentrations in peripheral circulation upon CNS hypoxic-ischemic pathologies in 14 months demonstrated the most significant reduction of the parameters in patients with paroxysmal syndrome, the change being less marked in examinees with EBD and psycho-speech disorder. Concentrations of TNF-α and IL-1 were found decreased most significantly in patients with psychomotor retardation to be 1.9 and 2.9 times higher than in those of the control group, respectively.

**Conclusion**

Reactivity of cytokine and interleukin links of immune systems was found varying in newborns and infants with CNS hypoxic-ischemic pathology, accompanied by psychomotor retardation, a psycho-speech disorder, EBD and paroxysmal syndrome. At admission irrespectively of a syndrome type in the patients with CNS hypoxic-ischemic pathology as compared with the patients in the control group circulating TNF-α and IL-1 were found increased by 3.4-4.1 and 6.4-7.9 times, respectively. Release of S100 protein and NSE seems to be the underlying mechanism for enhancement of synthesis and/or release of TNF-α and IL-1 into circulation of patients with CNS hypoxic-ischemic pathology.

**References**

Barashneva, Yu., 2001. Perinatal neurology. [Perinat'nal'naja nevrologija], in Russian, Moscow: Triada-x


