ENGLISH VERSION: APOPTOSIS MARKERS IN NEWBORN CHILDREN WITH MILD HYPOXIC DAMAGE OF CNS*

D.H. Posternak

SI «Luhansk State Medical University» of Ministry of Public Health of Ukraine, Ukraine, Rubizhne, Luhansk region.

Perinatal hypoxic damage of CNS in newborns is the leading reason of high mortality and disability at children's age. However, there are difficulties in diagnostics, therapy and forecasting of result of hypoxemic injuries of the brain in newborn children. We have studied apoptosis markers in blood (level of the circulating fragmented DNA and DNA fragmentation in lymphocytes) in 36 full-term newborn patients with mild perinatal hypoxic damage of CNS whose gestational age was from 36 to 41 weeks. The control group included 20 healthy full-term children. We defined severity of hypoxic damage of the central nervous system, using the anamnesis of a disease, Apgar scale, clinical criteria, change of the neurologic status according to N. P. Shabalov, as well as neurosonography. Investigation phases of newborns were at the first, third and seventh day. In patients with mild hypoxic damage of the central nervous system the unidirectional dynamics of changes in all studied apoptosis markers was noted. Hence, the maximum growth of the circulating fragmented DNA by 8.6% and increase in level of DNA fragmentation in lymphocytes by 1.6 times were noted at the 3rd day of research. At the last stage, the tendency to decrease in indicators and return to initial figures was observed. It was followed by improvement of the neurologic status of newborns. Thus, dynamics of change in markers of apoptosis such as level of the circulating DNA and DNA fragmentation in lymphocytes in healthy newborns, demonstrates adaptation of patient's body to the postnatal stress by the 7th day. Meanwhile, the unidirectional changes of levels of the studied indicators in newborns with mild hypoxic damage of CNS are reflected by the severity of brain damage, and also indicates adequacy of the chosen methods for the detection of apoptosis.

Keywords: hypoxia, newborn children, fragmented DNA, DNA fragmentation in lymphocytes, damage of CNS.

In the last decades, perinatal hypoxic damage of CNS in newborns is the leading reason of high mortality and disability at children's age [2, 8]. However, there are difficulties in diagnostics, therapy and forecasting of result of hypoxemic injuries of the brain in newborn children [1, 6].

Studying of such markers of apoptosis as size of the circulating fragmented DNA [3, 7] and DNA fragmentation level in lymphocytes of newborn children's blood is of great practical interest [4, 5].

Materials and methods

We have studied apoptosis markers in blood (level of the circulating fragmented DNA and DNA fragmentation in lymphocytes) in 36 full-term newborn children with mild perinatal hypoxic damage of CNS. The gestation age of patients was from 36 to 41 weeks. The control group included 20 healthy full-term children.

We defined severity of hypoxic damage of the central nervous system, using the anamnesis of the disease, Apgar scale, clinical criteria, classification of the neurologic status by N. P. Shabalov [2], and also methods of diagnostics (neurosonography).

Investigation phases of newborns were at the first, third and seventh day.

For definition of the circulating fragmented DNA and DNA fragmentation in lymphocytes we used the method based on color reaction with diphenylamine reagent in modification of the prof. I.A. Komarevtseva et al. (allocation is carried out in the lizis-buffer pH = 8).

Results and discussions

In healthy newborns of control group, the level of the circulating fragmented DNA in blood (Tab.1) decreased by the 7th day of neonatal period.

Table 1.
The level of the circulating fragmented DNA in blood of newborns with mild perinatal hypoxic damage of CNS.

Groups of observation	1st day	3rd day	7th day
Control group	16.4 ± 3.1 %	14.4 ± 2.8 %	13.8 ± 3.2 %
Group with mild HD of CNS	29.6 ± 3.9 %*	38.2 ± 4.2 %*	30.4 ± 3.3 %*

*Note: * - p < 0.05 – in comparison with control group;*
*** - p < 0.05 – in comparison with the previous stage*

The maximum growth of the circulating fragmented DNA was registered at the 3rd day by 8.6%, which exceeded basic data, in children with mild hypoxic damage of CNS. At the last stage of examination, the tendency of decrease and return to initial figures was observed.

Dynamics of level of DNA fragmentation in lymphocytes (Table 2) was similar to the level of the circulating fragmented DNA in blood in healthy newborns of control group. The studied marker also decreased by the 7th day of observation.

Table 2.
The level of DNA fragmentation in lymphocytes in blood of newborns with mild perinatal hypoxic damage of CNS.

Groups of observation	1st day	3rd day	7th day
Control group	10.8 ± 1.6 %	7.4 ± 1.8 %	4.6 ± 1.7 %
Group with mild HD of CNS	19.5 ± 2.9 %*	32.2 ± 2.4 %*,**	20.4 ± 2.1 %*,**

*Note: * - p < 0.05 – in comparison with control group;*
*** - p < 0.05 – in comparison with the previous stage*

In patients with mild hypoxic damage of the central nervous system the unidirectional dynamics of changes of all studied markers was noted. Hence, at the 3rd day

* *To cite this English version: D.H. Posternak. . Herasymenko, H.V. Nevoit. Apoptosis markers in newborn children with mild hypoxic damage of CNS // Problemy ekologii ta medytsyny. - 2015. - Vol 19, № 5-6. - P. 19–20.*

the level of DNA fragmentation in lymphocytes increased by 1.6 times, and at 7th day it decreased.

In newborns with mild perinatal hypoxic damage of CNS at the 3rd day, the increase of apoptosis phenomena was registered. It coincided with deterioration in the neurologic status which was manifested as transient syndrome of excitement and/or oppression of CNS. The syndrome of excitement was characterized by locomotor concern, tremor, starts, unmotivated shout, moderate tachycardia and tachypnoe. To oppression symptoms we classified muscular hypotonia or dystonia, a hypokinesia, fast exhaustion of congenital reflexes and decrease in cerebral activity. Neurosonography changes were absent. The increase of all studied apoptosis markers was noted.

At the last investigation phase we observed positive dynamics which was manifested in reduction of apoptosis intensity. It corresponded to reduction of level of the studied indicators with a tendency to return to initial figures. Thus, at the 7th day of observation, the level of the circulating fragmented DNA, in comparison with the 3rd day, was by 7.8% lower (Table 1), and the number of DNA fragmentation in lymphocytes decreased by 1.6 times (Table 2).

The criteria of neurologic improvement at children were as follows: restoration of adequate level of consciousness, improvement of reflex functions, restoration of the emotional sphere (reactions to external irritants, emergence of spontaneous physical activity, active sucking of a pacifier, manifestation of hungry concern).

Conclusions

Dynamics of change of markers of apoptosis such as level of the circulating DNA and DNA fragmentation in lymphocytes in healthy newborns of control group, demonstrates adaptation of patient's body to the postnatal stress by the 7th day.

The unidirectional changes of levels of the studied indicators in newborns with mild hypoxic damage of CNS in the early neonatal period are reflected by the severity of brain damage, and also indicates adequacy of the chosen methods for the detection of apoptosis.

References:

1. Neonatologiya: natsional'noye rukovodstvo. N.N. Volo¬din, red. M.: GEOTAR-Media, 2013: 896 s.
2. Pal'chik A.B., Shabalov N.P. Gipoksicheski-ishemicheskaya entsefalopatiya novorozhdennykh. 4-ye izd., ispr. i dop. – M.: MEDpress-inform, 2013. – 288 s.
3. Tamkovich S.N., Vlasov V.V., Laktionov P.P. Tsirkuliruyushchiye DNK krovi i ikh ispol'zovaniye v meditsinskoy diagnostike. Molekulyarnaya biologiya 2008; 42: 1: 12-23.
4. Ioannou YA, Chen FW. Quantitation of DNA fragmentation in apoptosis. Nucleic Acids Res 1996; 24:992–3.
5. Manoj A, Ramachandra RK, Vishnu BB, Venkatesh C, Bobby Z. DNA damage in perinatal asphyxia using micronucleus assay. Curr Pediatr Res 2011;15(1):5–9.
6. Polat M., Simşek A., Tansuğ N. et al. Prediction of neurodevelopmental outcome in term neonates with hypoxic-ischemic encephalopathy // Eur. J. Paediatr. Neurol. – 2013. – Vol. 17, №3. – P. 288-293.
7. Rainer TH, Wong LKS, Lam W, Yuen E, Lam NYL, Metrewel C, et al. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. Clin Chem 2003; 49:562–9.
8. Soliman A.M., Al-Gendy R.A., Abdel-Moety H. Hypoxicischemig encephalopathy in term neonates: early biochemical indicators // Australian J. Basic and Applied Sci. – 2011. – № 5. – P.82-87.

Матеріал надійшов до редакції 23.02.2016