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NEUROSPECIFIC MARKERS OF EARLY DIAGNOSIS AND PREDICTION OF PERINATAL CNS LESION IN PREMATURE NEWBORNS

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Abstract: The aim of the study was to study the levels of neurospecific proteins NSE, BDNF, VEGF in newborns, depending on the gestation period.

Research materials and methods: 171 newborns with different gestational ages were examined. All children were divided into 3 groups: the 1st main group consisted of 61 premature newborns with gestational age from 32 to 33+6 weeks (33.5±0.11), the 2nd group (comparison) included 60 premature newborns – 34-36+ 6 weeks (35.0±0.11). The control group consisted of 50 newborns with a gestational age of 37+6-40 weeks, whose body weight corresponded to the gestation period. All newborns underwent a thorough obstetric and gynecological anamnesis, a general clinical examination, and an assessment of the state of the nervous system on the Thompson scale "Assessment of the severity of central nervous system damage". In addition, all newborns underwent a study of neurospecific proteins: determination of the concentration of neurospecific enolase (NSE), brain neurotrophic factor (BDNF), vascular endothelial factor (VEGF). Statistical processing of the results was performed by statistical methods using standard ("MS Excel-XP") software tools.

Results: Our research has shown that the diagnosis of neurological disorders in the neonatal period by determining the concentration of neurospecific factors is important for predicting the formation of disabling processes. The results of the study can serve as a supplement in solving the issue of early diagnosis and prognosis of perinatal CNS damage in premature infants.

Keywords: premature newborn, perinatal central nervous system lesion, neurospecific factors, Thompson scale, neurospecific enolase (NSE), brain neurotrophic factor (BDNF), vascular endothelial factor (VEGF),

The developing brain of a newborn is extremely sensitive to the effects of hypoxia, which not only causes focal damage to brain tissue, but also delays the development of the vascular system, disrupting cellular differentiation. According to foreign authors, the frequency of hypoxic lesions in full-term newborns is no more than 0.6%, while in premature infants it varies widely up to 30% [2,4,5].

Early diagnosis of perinatal lesions of the nervous system presents significant difficulties due to immaturity of brain structures in premature infants, instability of clinical symptoms and changes in phase, adaptive states. The incompleteness of the cortical localization of CNS functions is the reason that often prematurely born children lack focal symptoms even with significant changes in brain structures [1,3,9,10].

In recent years, the attention of researchers has been attracted to the study of the role of neuroglial interactions in the pathogenesis of ischemic brain damage,

primarily in the aspect of the interaction of neurons and astrocytes, especially the energy metabolism of neurons [6,11,12].

With hypoxic-ischemic brain damage, there is an increase in the permeability of the blood-brain barrier (BBB), which leads to the entry of neuron-specific proteins into the blood, which are markers for diagnosing the severity of the lesion and the nearest prognosis of CNS damage. According to many authors, an increase in NSE in premature newborns who have suffered asphyxia is an unfavorable factor in relation to the prognosis of further psychomotor development. Of particular interest is the relationship between the content of brain neurotrophic factor (BDNF) and vascular growth factor (VEGF). According to a number of authors, these markers directly correlate with the formation of severe posthypoxic structural changes in the brain in newborns [8,9,12].

The brain neurotrophic factor BDNF attracts special attention of researchers due to its neurotrophic effect, having a stimulating effect on the growth of new neurons and suppression of cell apoptosis [12,13,15].

Similar results are presented for vascular endothelial growth factor (VEGF), while some studies have revealed an important role of this protein in neurogenesis and neuroprotection in the developing brain [5,13,14,15].

Many researchers have repeatedly stressed that the earlier the correction of perinatal lesions of the nervous system in children is detected and started, the fewer consequences are observed during the development of the child [13,14,15]. In this connection, one of the most pressing problems is the identification of reliable and objective predictors of this condition.

The aim of the study was to study the levels of neurospecific proteins NSE, BDNF, VEGF in newborns, depending on the gestation period.

Materials and methods of the study: 171 newborns with different gestational ages have been examined and divided into 3 groups: the 1st main group consisted of 61 premature newborns with gestational age from 32 to 33+6 weeks (33.5±0.11), the 2nd group (comparison) included 60 premature newborns - 34-36+6 weeks (35.0±0.11). The control group consisted of 50 newborns with a gestational age of 38-40 weeks, whose body weight corresponded to the gestation period.

The criteria for inclusion in the study groups were newborns with acute cerebral insufficiency. All newborns underwent a thorough obstetric and gynecological anamnesis, a general clinical examination, and an assessment of the state of the nervous system on the Thompson scale "Assessment of the severity of central nervous system damage". In addition, all newborns underwent a study of neurospecific proteins: determination of the concentration of neurospecific enolase (NSE), brain neurotrophic factor (BDNF), vascular endothelial factor (VEGF), which was determined by the immune- enzyme blood test on the ELISE enzyme immunoassay. Blood was taken from the ulnar vein in a volume of 2 ml. The obtained data were processed by statistical methods using standard ("MS Excel-XP") software tools that ensure the effective use of mathematical logic and statistical analysis methods.

Results. In the occurrence of pathology of the neonatal period, the state of the mother's health plays a great role immediately before and during pregnancy. We have

studied the peculiarities of obstetric - gynecological and somatic status of mothers of the examined groups of newborns.

The results of our studies showed that the course of the antenatal period in premature infants in 90.1% (1st and 2nd groups) is burdened, whereas among full-term infants (3rd group), the burden was recorded 3.2 times less often (28%; P<0.01).

Also , it should be noted that the lower the gestation period at birth, the higher the frequency of the burden of the antenatal period in premature infants (Fig.1.), which is confirmed by the correlation relationship (P<0.05).

With an increase in the gestation period, the frequency of antenatal complications decreases, and the strength of the correlation relationship decreases, so if at the time of gestation 32-33+6 weeks there is a strong r=-0.859 ($\chi 2$=7.562; P<0.05), then with a gestation period of 38-40 weeks, a weak inverse relationship is recorded equal to r=-0.231 ($\chi 2$=0.562; P>0.05)

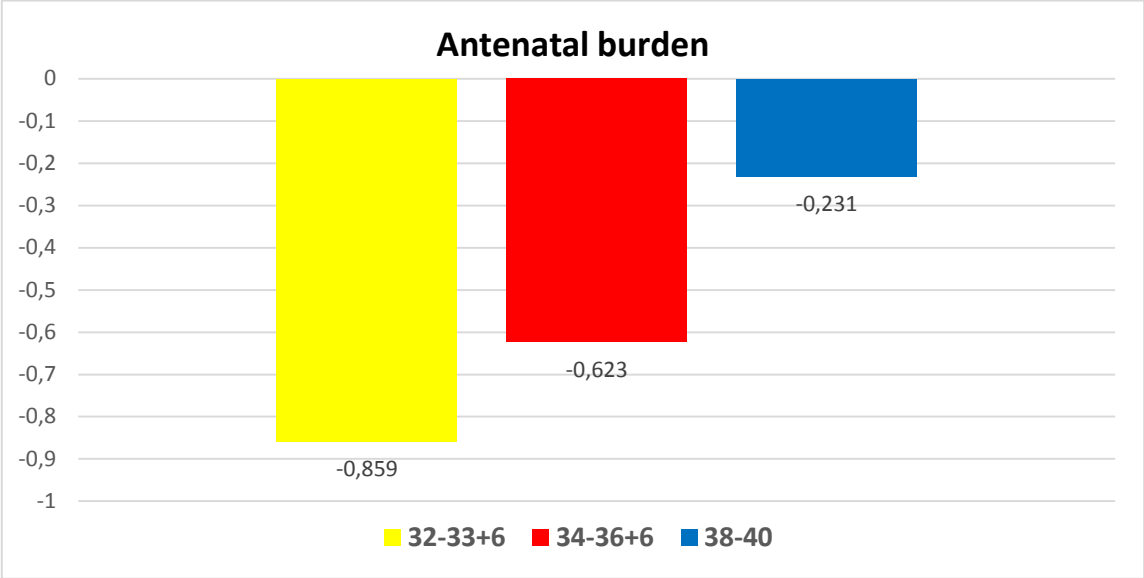

Fig. 1. Indicators of the correlation relationship between the presence of the burden of the antenatal period and the timing of gestation at birth among the examined children

A thorough analysis of the intranatal period was burdened in 91.8% of mothers of group 1, 91.7% of mothers of group 2 and 38% of mothers of group 3. The reliability of these high indicators of the burden of the intranatal period in groups 1 and 2 in relation to group 3 (P<0.05, respectively) has been established.

The influence of the frequency of the burden of the intranatal period on the birth of premature babies is confirmed by the correlation relationship (Fig. 2).

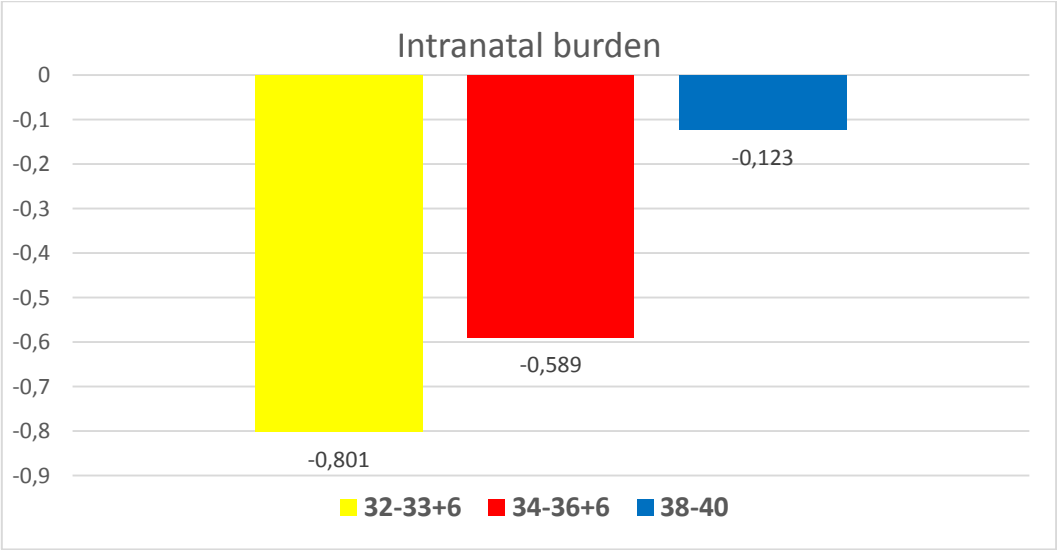

Fig. 2. Indicators of the correlation relationship between the presence of the burden of the intranatal period and the timing of gestation at birth among the examined children

As can be seen from Fig.2. at gestational age 32-33+6 weeks, there is a strong inverse correlation equal to r=-0.801 ($\chi 2$=6.895; P<0.05), whereas with a gestation period of 38-40 weeks, a weak inverse relationship is recorded - r=-0,123 ($\chi 2$=0.485; P>0.05)

We assessed the risk factors of premature birth of newborns depending on the course of pregnancy with the calculation of attributive and relative risk. As can be seen from Table 1, a high relative risk of having children with low body weight in pregnant women was observed in anemia (OR=8.17), multiple pregnancies (OR=6.58), feto-placental insufficiency (OR=6.46). The risk ratio for manifestations of proteinuria and preeclampsia ranged from 4.86 to 5.28, which indicated a direct relationship between these conditions and the severity of their influence. Pregnant women with such complications were 3 times more likely to have babies born prematurely compared to pregnant women with normal kidney function and blood pressure. Maternal infection also had a high effect on the birth of premature babies (OR=6.23).

Assessment of risk factors for the birth of premature newborns depending on the course of pregnancy

Risk factor	**Attributive risk**	Coefficient of relative risk
Anemia	0,88	8,17
Multiple pregnancy	0,84	6,58
Placental insufficiency	0,81	6,46

Proteinuria	0,79	4,86
Arterial hypertension	0,87	9,41
Infection rate	0,86	6,23
Threat of rupture of fetal membranes	0,76	4,23
Preeclampsia	0,84	5,28

The analysis of risk factors for the birth of premature babies confirmed the multifactorial nature of their occurrence.

The examined children after childbirth were evaluated on the Apgar scale at the 1st and 5th minute. If necessary, the third examination was performed at the 10th minute. The results of our research showed that among premature infants of both groups, the Apgar score at the 1st minute was significantly lower than at the 5th. So, in the 1st group, the average score on the Apgar scale at the 1st minute of life was 5.0± 0.17 points, at the 5th minute of life 6.3± 0.14 points, and in the 2nd group at the 1st minute of life was 6.2± 0.12 points, at the 5th minute of life 7.3±0.10 points.

After birth, all premature babies were admitted to the intensive care unit and the Neonatal pathology department.

When assessing the duration of hospital stay (Fig. 3), it was found that statistically children from group 1 spent significantly longer both in intensive care - 19.3±2.4 days ($p<0.05$) and in the neonatal pathology department - 41.4±1.9 days ($p<0.001$).

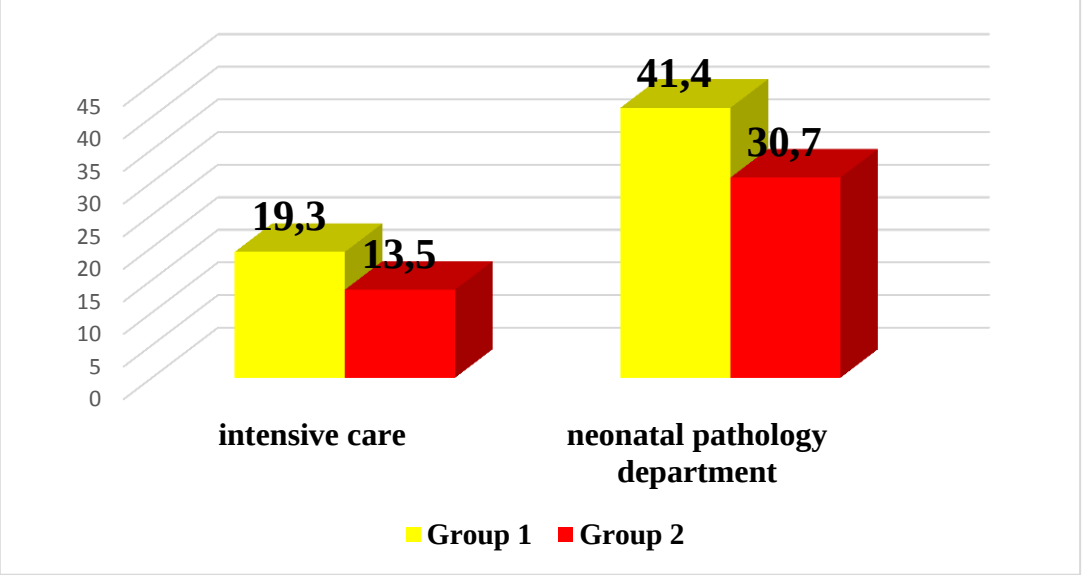

Fig. 3. The number of days spent by premature newborns in the hospital.

It is evident that premature babies born during gestation 32-33+6 weeks, need longer treatment than children born at 34-36+6 weeks gestation both in the intensive care unit and in the neonatal pathology department. Accordingly, this contingent of children requires the greatest expenses in nursing, and our data also show this.

When analyzing the neonatal period, it was found that each child had respiratory distress syndrome (RDS), which required respiratory support. According

to our data, 26 (42.6%) children of group 1 at birth and 13 (21.7%) children of group 2 (P<0.05) needed hardware support from the first minute of life.

Signs of perinatal hypoxic-ischemic brain damage of the 2nd degree were registered in groups 1 and 2, respectively, in 59% (36/61) and 31.7% of children (19/60), but the 3rd degree was 2.2 times more. Grade 3 VVC was 2.5 times more often diagnosed in premature infants of group 1 than in group 2 (7.2% (4/61) and 3.3% (2/60), respectively; P<0.05). Significantly more often periventricular leukomalacia occurred in premature infants of group 1 than in children of group 2 (p<0.05).

Thus, signs of cardio-respiratory maladaptation were present in 42.6% (26/61) of premature newborns of the 1st observation group, and in 30.0% (18/60) in the 2nd group, but were more pronounced in newborns of the first group, which could mediate the development of VVC from the first day of life.

In all premature newborns, jaundice had a conjugation character. Newborn children of groups 1 and 2 had a longer jaundice period. Jaundice in premature infants occurred in 84.3% of cases in group 1 and in 82.5% of cases in group 2.

Severe anemia at birth prevailed in infants of group 1 (p<0.01). The high frequency of diseases of the nervous and respiratory system in premature infants (RDS, cerebral ischemia) is due to the more pronounced immaturity of these systems at the time of birth. RDS was registered in 94.0% of group 1 children and 67.0% of group 2 newborns.

A score assessment of the neurological condition of the examined premature newborns with PPCNS on the Thompson scale has been conducted (Fig. 4). According to this scale, depending on the amount of points scored, newborns with scores from 1 to 10 have a mild degree, from 11 to 14 an average degree, and children with a total of 15 to 22 points they have a severe degree of perinatal damage to the central nervous system. Premature newborns in group 1 scored an average of 10.6± 0.36 points on the Thompson scale, in group 2 - 8.83± 0.36 points, which is significantly lower than the indicators of group 1 (P<0.001).

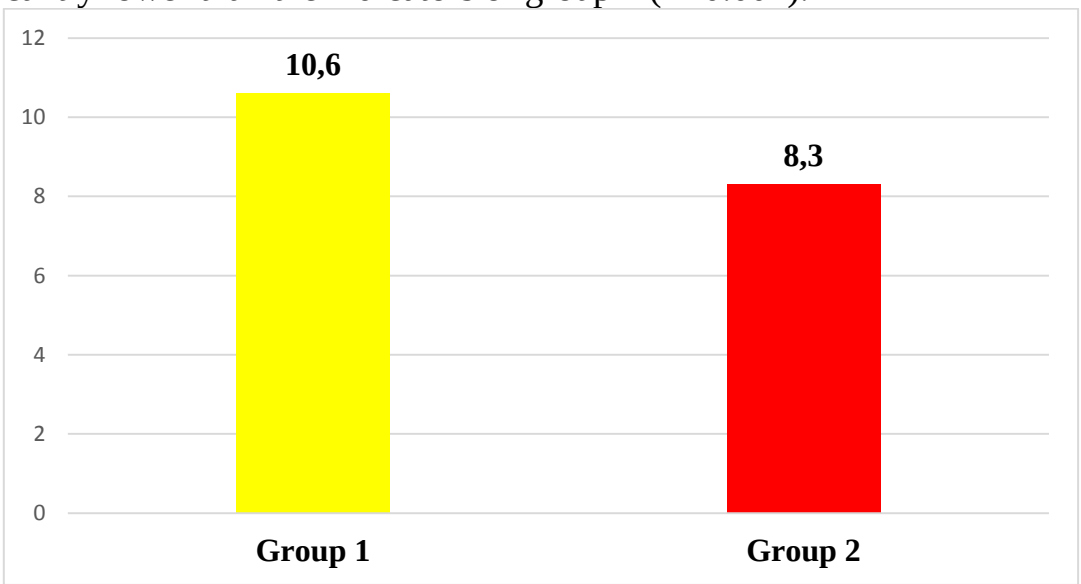

Fig. 4. Score in the examined premature newborns with PPCNS on the Thompson scale.

Depending on the identified clinical signs, three groups were identified according to the severity of the CNS lesion (Table 2).

Table 2.

Distribution of the examined newborn children depending on the indicators of the Thomson scale

Severeness degree according to Thompson' scale	Group 1 (n=61)			Group 2 (n=60)		
	Abs	%	Average score	Abs.	%	Average score
Mild degree	25	41,0	8,0±0,42	44	73,3	7,6±0,32
Moderate degree	33	54,1	12,0±0,17	16	26,7*	12,3±0,31
Severe degree	3	4,9	16,0±1,2	-	-	-

Note: * - reliability of data between groups (P≤0.05)

As can be seen from the table, a mild degree of CNS damage was observed more often in newborns of group 2 (73.3% (44) versus 41.0% (25), respectively, of group 1), but the data did not reach reliable values. Moderate severity was significantly more often recorded in group 1 (P≤0.05). Severe degree was observed in 4.9% (3) premature infants of group 1, in group 2, severe CNS lesion was not recorded.

Thus, the Thompson's scale makes it possible to more accurately describe the neurological status and its deviations even in premature newborns. The results of summing up the scores allow us to determine the severity of the central nervous system lesion.

At the next stage of our work, we studied the average levels of neuron-specific proteins NSE, BDNF, VEGF among the examined newborns (Fig. 5.). When studying the data presented in premature infants, an increase in NSE and VEGF indicators (1.8 times and 2.6 times, respectively, relative to the control) was revealed against the background of low BDNF indicators (1.6 times from the control).

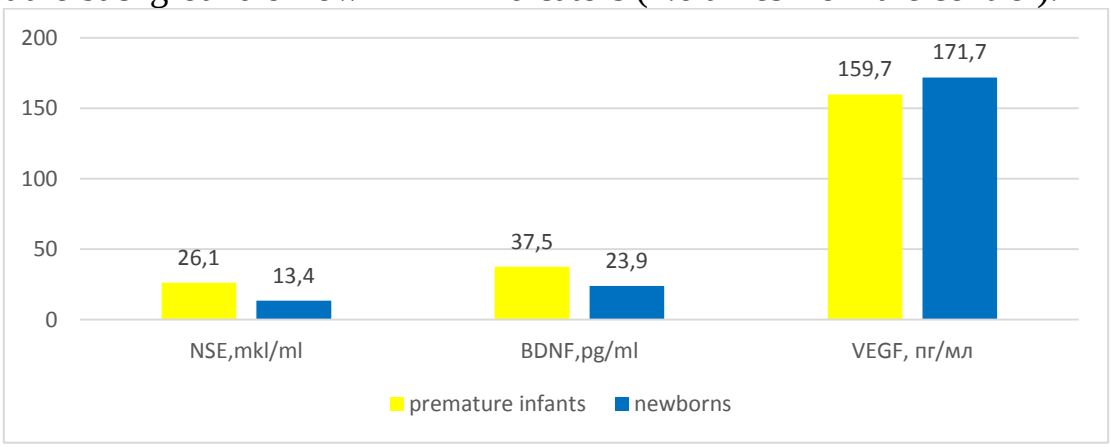

Fig. 5. Average levels of neurospecific proteins NSE, BDNF, VEGF among the examined newborns

In premature infants, BDNF indices were higher than in full-term newborns, due to the brain's response to stress at birth, the impact of a combination of external factors and the need for rapid formation of new polysynaptic connections. The results obtained do not contradict the literature data on the increase in BDNF formation during the recovery of brain damage [11]. An increase in the BDNF index in premature infants during the neonatal period indicates both the restoration of damaged areas of the brain and the physiological maturation of morphological structures.

A strong inverse correlation was obtained between the concentration of BDNF in blood serum and the formation of hypoxic-ischemic encephalopathy (r=0.789; P<0.01; Fig. 6).

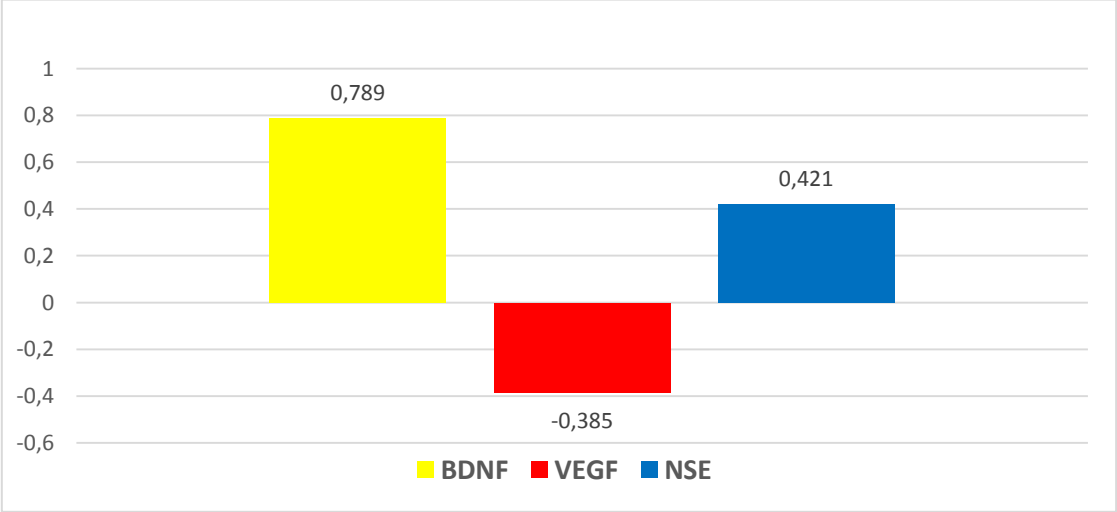

Fig. 6. Indicators of correlation between the level of neuron-specific proteins NSE, BDNF, VEGF and the development of HIE

VEGF values in premature infants significantly exceeded those in the group of full-term infants (P<0.05). High VEGF values in premature infants reflect the processes of intensive angiogenesis aimed at restoring blood supply in the areas of cerebral ischemia and damage to the vessels of the germinal matrix.

The NSE values in premature infants exceeded those in children of the control group, which confirms intrauterine hypoxic suffering of the premature infant and damage to brain structures.

When studying the average values of neuron-specific proteins NSE, BDNF, VEGF, depending on the gestation period (Table. 3), it was found that newborns born at gestation from 32 to 33+6 weeks (group 1) had lower VEGF values (149.8±5.6pg/ml versus 159.2±4.2 pg/ml; P<0.05) against the background of high NSE values (27.8±2.1 µl/l and 22.5±2.3 µl/l; P<0.05) and BDNF (44.5±2.3 pg/ml and 31.6±1.8 pg/ml; P<0.05) in relation to the indicators of newborns born at gestation from 34-36+6 weeks (group 2) and the control group NSE – 13.4±0.8 (P<0.01); VEGF – 171.7±5.6 pg/ml (P<0.05) and BDNF – 23.9±2.8 pg/ml (P<0.05)).

Table 3.

The level of the content of neurospecific proteins NSE, BDNF, VEGF depending on the gestation period in newborns

	Group 1	Group 2	Group 3
NSE, мкл/л	27,8±2,1***	22,5±2,3**^	13,4±0,8
VEGF, пг/мл	149,8±5,6*	159,2±4,2*^	171,7±5,6
BDNF, пг/мл	44,5±2,3**	31,6±1,8*^^	23,9±2,8

Note: * - reliability of data in relation to the control group (* - P<0.05; ** - P<0.01); ^ - reliability of data in relation to the control group (^ - P<0.05; ^^ - P<0.01)

The data of the correlation analysis indicate a strong correlation between the indicators of neuron-specific proteins NSE, BDNF, VEGF with the gestational age of newborns (r=0.784; P<0.01; r=0.632; P<0.01 and r=-0.711; P<0.01, respectively; Fig. 7)

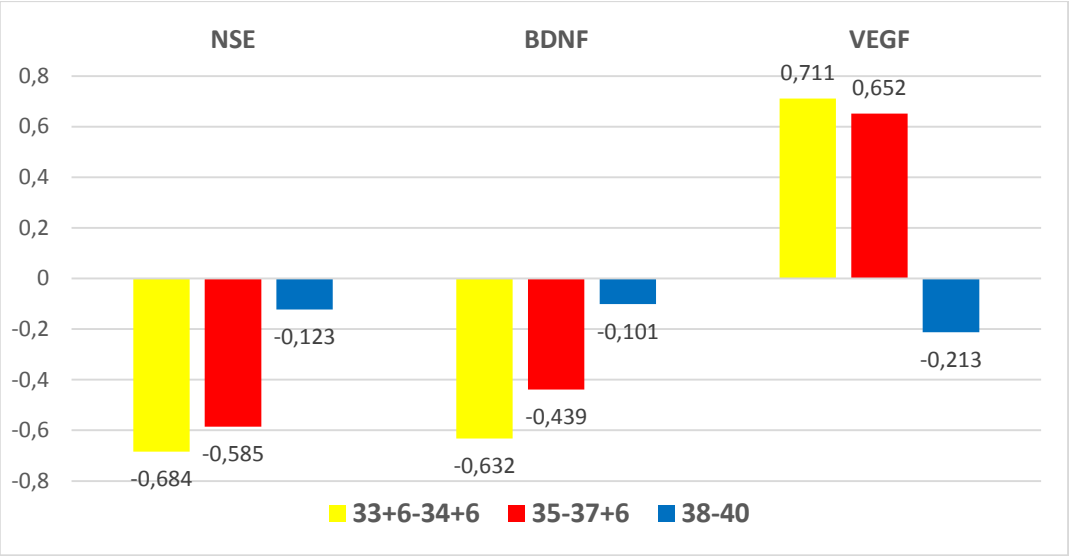

Fig. 7. Indicators of correlation of the level of neurospecific proteins NSE, BDNF, VEGF from the gestation period at birth among the examined children

When studying the role of neuron-specific proteins NSE, BDNF, VEGF in the pathogenesis of the development of PPCNS, we found that these indicators vary depending on the severity of the central nervous system lesion on the Thompson scale (Table 4).

Table 4.

Indicators of neuron-specific proteins NSE, BDNF, VEGF among the examined premature newborns, depending on the severity of the central nervous system lesion according to Thompson

Indications	Control group	PPCNS degree		
		mild	moderate	severe
NSE, мкл/л	13,4±0,8	14,1±4,1	21,6±6,2*^	27,49±4,3**^^

VEGF, пг/мл	171,7±5,6	169,3±7,7	157,9±13,3*	149,0±9,8*
BDNF, пг/мл	23,9±2,8	25,3±2,8	33,3±2,1*	42,7±5,3**^

Note: * - reliability of data in relation to the control group (* - P<0.05; ** - P<0.01); ^ - reliability of data in relation to the control group (^ - P<0.05; ^^ - P<0.01)

As can be seen from the presented data, in children with mild central nervous system damage according to Thomson, the parameters of neuron-specific proteins NSE, BDNF, VEGF did not significantly differ from the parameters in the control group. However, the content of NSE and BDNF tends to increase (14.1±4.1 µl/l versus 13.4±0.8 µl/l; 25.3±2.8 pg/ml versus 23.9±2.8 pg/ml), while the level of VEGF tends to decrease, but at the same time, reliability has not been established (P>0.05). With a moderate degree of damage to the central nervous system according to Thompson, there was a more pronounced imbalance in relation to the data with a mild degree and to the control group, especially in terms of the NSE level. The severe degree of CNS lesion was characterized by a significant increase in NSE both in relation to the mild degree and in relation to the control group (P<0.05-0.01).

The established deviations in the studied laboratory parameters can to some extent explain the disturbances in the formation of certain structures of the central nervous system, the course of regenerative processes in the nervous tissue in response to its hypoxic damage, recognizing the important role of neurotrophic factors in the mechanisms of the pathogenesis of brain hypoxia.

Cross-correlation analysis (Fig.8) confirmed the relationship of the indicators of neuron-specific proteins NSE, BDNF, VEGF with the severity of damage to the Thompson nervous system, which was most pronounced in severe cases (r=0.652; P<0.01; r=0.589; P<0.01 and r=-0.518; P<0.01, respectively).

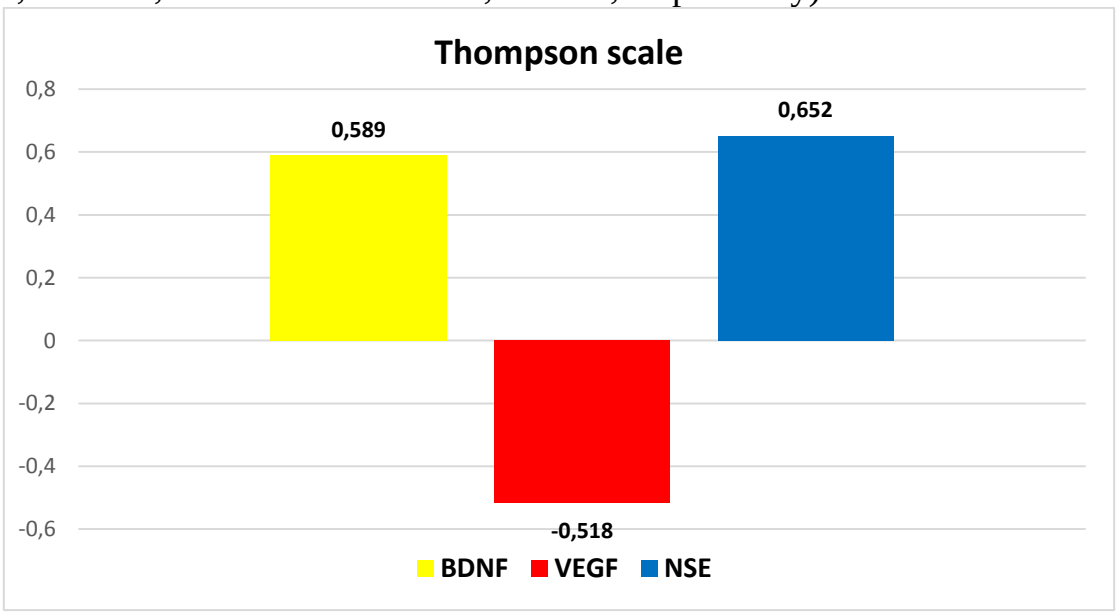

Figure 8. Indicators of correlation between the level of neuron-specific proteins NSE, BDNF, VEGF and Thompson scale indicators

Thus, the data obtained indicate a limitation of BDNF synthesis and insufficient provision of reparative processes in the damaged brain in premature

infants, especially with severe Thompson's degree. A multiple decrease in the concentration of VEGF demonstrates a decrease in the restoration of damaged and the formation of new vessels and reflects a high degree of brain destruction in premature infants with PCNS. Elevated NSE levels have an unfavorable prognostic value in premature infants with CNS lesions. Diagnosis of neurological disorders in the neonatal period by determining the concentration of neuron-specific factors is important for predicting the formation of disabling processes. The results of the study can serve as a supplement in solving the issue of early diagnosis and prognosis of perinatal CNS damage in premature infants.

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