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CLINICAL AND MORPHOLOGICAL CORRELATES OF PINEAL GLAND FUNCTION IN INFANTS WITH EXTREMELY LOW BODY WEIGHT

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Summary

Introduction. *The functional activity of the pineal gland plays a dramatic important role in the adaptation to postnatal life and in the pathogenesis of the most common perinatal pathology of premature infants.*

The aim of the study. *To determine the morphofunctional features of the pineal gland in premature infants with extremely low body weight.*

Material and methods. *In 46 preterm infants with extremely low birth weight, the level of melatonin metabolite in urine 6-sulfoxymelatonin was determined at first day of life. The 20 dead infants underwent macro- and microscopic examination of the pineal gland using the immunohistochemical method.*

Results. *All preterm infants with extremely low birth weight had perinatal pathology, which led to death in 20 of them. Urinary excretion of the metabolite melatonin 6-sulfoximelatonin in preterm infants with extremely low birth weight in the first day of life, which had fatal consequences, significantly reduced compared with surviving children, indicating depletion of functional activity of the pineal gland and may be as a marker of adverse course of the neonatal period. Morphologically, in the pineal gland of premature infants with extremely low body weight there is an increase in morphofunctional activity of pineal cells. This is confirmed by morphometric data and increased expression of MelanA and S100 in immunohistochemical studies. Macro- and microscopic data suggest that extrauterine existence in distress conditions accelerates the differentiation of the pineal gland (depletion) and indicates the presence of damage to glandular tissue, which in turn reduces the synthesis of melatonin and its mediated metabolite 6 – sulfoxymelatonin in urine.*

Conclusions. *Decreased urinary excretion of 6-sulfoximelatonin in preterm infants with extremely low birth weight in the first day of life and mophological changes in the pineal gland of deceased children indicate depletion of functional activity of the pineal gland in conditions of perinatal pathology.*

Key words: *Melatonin; Prematurity; Pineal Gland.*

Introduction

In the pathogenesis of the most common pathology of preterm infants, an extremely important role belongs to the neuroendocrine system, on the functional state of which depends the adaptation to postnatal life [1]. In recent years, more and more attention has been focused on the antioxidant and anti-inflammatory effects of the epiphysis hormone melatonin [2]. To date, insufficient data have been accumulated on the role of the epiphysis in the adaptation and compensation of perinatal pathology and little attention has been paid to studying the features of epiphysis function in newborns with extremely low birth weight (ELBW) [3, 4]. To develop new methods of diagnosis, therapy, and prevention of perinatal pathology, the search for biological regularities and knowledge about the features of brain epiphysis function in prematurely born infants is necessary [5]. A clear understanding of the peculiarities of adaptation in the organization and compensation of structural and functional changes in premature infants is highly relevant. Promotion of medically supported compensation processes in nursing preterm infants based on the features of epiphyseal functions and morphological properties is one of the important ways to reduce neonatal morbidity and mortality [6].

The aim of the study

To determine urinary 6-sulfoxymelatonin levels in the first day of life in preterm infants with ELBW and to perform pathomorphological examination of epiphysis samples from deceased infants followed by clinical and morphological comparisons.

Material and Methods

Forty-six preterm infants with ELBW (body weight from 500 g to 999 g) (Bioethics Commission Protocol #8 dated 10/21/2021.) were followed up. The level of functional activity of the pineal gland was studied by quantifying the level of 6 -sulfoxymelatonin (6 - SM) in urine on the first day of life by enzyme immunoassay using the BÜHLMANN 6-Sulfatoxymelatonin ELISA test (BUHLMANN Diagnostics Corp, USA).

To assess structural and functional changes in the pineal gland in 20 prematurely born infants who died in the early neonatal period we performed morphological and histological examination of pathological anatomical samples of epiphyses at the clinical laboratory of the Department of Pathological Anatomy at KMAPE (Head - I.I. Yakovtsova). For histological study of the epiphysis we used prepared fixed micro preparations of the gland, which were stained with hematoxylin and eosin. Morphometric characteristics (nuclear area, cell area, nuclear cytoplasmic index) were calculated.

For immunohistochemical study, the material was fixed with 10 % neutral formalin for 24 hours,

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embedded in paraffin, prepared 4 μm-thick sections that were applied to highly adhesive Super Frost glass and dried at 37°C for 18 hours. Demasking heat treatment was performed by boiling the sections in citrate buffer (pH 6.0). An UltraVision Quanto Detection Systems HRP Polymer (Thermo scientific) was used to visualize primary antibodies. DAB (diaminobenzidine) was used as a chromogen. The marker antibodies MelanA (Vitro, Spain) and S100 (Leica Biosystems Newcastle, UK) were used.

Exel for Windows and Statistica 7.0 for Windows software packages were used for statistical analysis. Check of data distribution for compliance to Gauss law was performed using Shapiro-Wilk's criterion. Median (Me); minimum and maximum values (min is minimum and max is maximum), 95% confidence interval (CI) were determined. Nonparametric Mann-Whitney U-criterion (MW test) was used to compare two independent samples. The relative risk index (RR) and its 95% confidence interval were used. Fisher's F-criterion was used to compare particles. The data obtained during statistical analysis were considered reliable at $p<0.05$.

Results and Discussion

The maternal course of pregnancy and delivery in infants with ELBW included the following conditions: premature rupture of membranes, 18 (39.1 %); risk of miscarriage, 13 (28.2 %); urogenital infections, 7 (15.2 %); multiple pregnancy, 12 (26.0 %); preeclampsia, 6 (13.0 %); cesarean section, 28 (68.0 %); and fetal distress, 13 (28.2 %). Clinical and demographic data of preterm infants with ELBW are presented in Table 1.

Table 1

Clinical characteristics of preterm infants with ELBW

Data	n (%)	95 % CI
Boys	16 (34,7)	21,3 – 50,2
Girls	30 (65,2)	497 – 78,6
Gestational age: ≤ 25 weeks 26 weeks 27 weeks 28 weeks 29 weeks	 4 (8,6) 4 (8,6) 7 (15,2) 25 (54,3) 8 (17,3)	 2,4 – 20,7 2,4 – 20,7 6,3 – 28,8 39,0 – 69,1 7,8 – 31,4
Respiratory distress syndrome	46 (100)	92,2 – 100,0
Anemia of prematurity	15 (32,6)	19,5 – 48,0
Hypoxic-ischemic encephalopathy	26 (56,6)	41,1 – 71,0
Congenital pneumonia	20 (43,4)	28,9 – 58,8
Intraventricular hemorrhage	22 (47,8)	32,8 -63,0
Sensory hearing loss	16 (34,7)	21,3 – 50,2
Neonatal sepsis	14 (30,4)	17,7 – 45,7
Severe asphyxia	26 (56,6)	41,1 – 71,0
Retinopathy of prematurity	20 (43,4)	28,9 – 58,8
Bronchopulmonary dysplasia	7 (15,2)	6,3 – 28,8
Necrotizing enterocolitis	10 (21,7)	10,9 – 36,3

When comparing the incidence of pathological conditions in the antepartum and intrapartum periods in preterm infants with ELBW who survived (n=26) and those who died (n=20), no significant differences were determined. There were no significant differences in gestational age among surviving and deceased children: 28 (min 25; max 29) weeks and 28 (min 24; max 28) weeks, respectively (MW test, p=0.4257). When comparing the frequency of pathological conditions in children who died, only a significant increase in the frequency of grade III-IV IVH was determined (9 of 12), whereas among the 10 surviving children only two children had grade III-IV IVH, the remaining 8 children had grade II IVH (RR = 3.8 95% CI 1.1 to 13.5), p=0.0433. There were no significant differences in the incidence of other pathological conditions in children with ELBW who died and those who survived.

The results of urine 6-SM analysis in the first day of life in preterm infants with ELBW demonstrated its significant reduction in those with lethal outcomes. In surviving infants, urinary 6-SM values were: 180.6 (min 22.0; max 501.0), 95% CI 180.66 to 352.14 pg/ml, and in infants who died: 65.5 (min 7.00; max 501.00), 95% CI 22.07 to 106.42 pg/ml (MW test, p=0.0006).

On macroscopic examination in infants, the epiphysis was predominantly semilunar and cone-shaped. Microscopic examination of the glands revealed pronounced signs of acute circulatory disorder in the form of sharp dilatation and fullness of capillaries with their ruptures and formation of microhematomas both in the parenchyma and in subcapsular parts (Fig. 1).

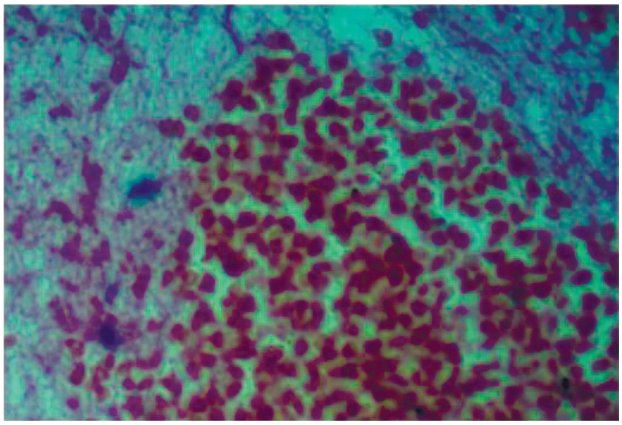

Figure 1. Microhematoma in the epiphyseal parenchyma of a neonate with extremely low body weight. Hematoxylin-eosin staining, x1000

Light microscopy showed predominantly dark pinealocytes with scanty cytoplasm in the form of a thin rim, multiple immature pinealocytes with a small dark nucleus. Dark pinealocyte nuclei possessed dark karyoplasm with a structureless mass of condensed chromatin. A small number of light cells with vacuolized cytoplasm and rounded or angular nucleus were detected. We noted a decrease in the area of actively functioning light cells (93.4 μm2), with a tendency for the nucleus to decrease (38.7 μm2), and the nuclear cytoplasmic index was 0.4. Light cells were observed not only in the center of the gland parenchyma, but also focally along the periphery, in some places under the capsule, a light zone was detected (Fig. 2).

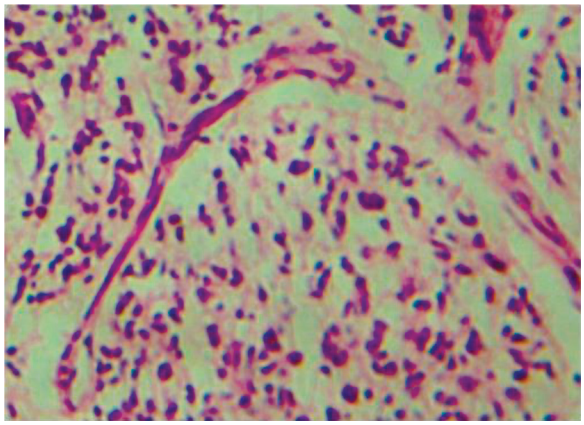

Figure 2. Light area under the pineal capsule of a neonate with extremely low body weight. Hematoxylin-eosin staining, x400

Some cells showed shadows instead of nuclei, abundant cytoplasm, in some cells cytolemma integrity disorder and even its disappearance were detected. Focal accumulations of fluid due to cell lysis were detected in the center of gland particles (Fig. 3).

Immunohistochemical examination demonstrated moderate expression of MelanA (Fig. 4) and moderate expression of S100 (Fig. 5) in light active pinealocytes. The moderate expression of these markers confirms the fact that in children with ELBW at birth, even those with an unfavorable course of the neonatal period, pinealocytes have functional activity, namely light cells. Light cells, unlike dark pinealocytes, are considered mature. So, the adaptive function of the light cells of the epiphysis is functional for extrauterine life in preterm infants with ELBW. Therefore, many other factors influence thanatogenesis and reduced pineal function.

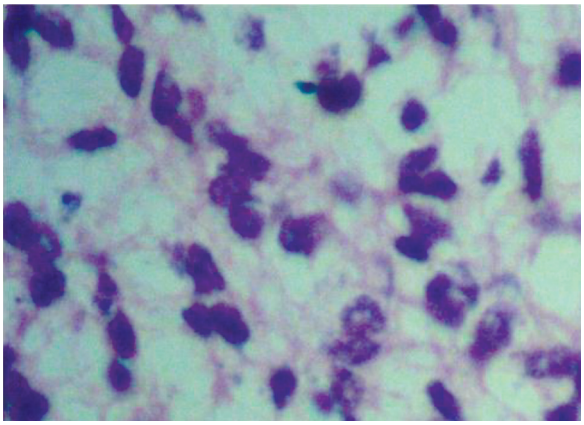

Figure 3. Plots of focal and complete lysis of pinealocytes in the epiphysis of a neonate with extremely low body weight. Hematoxylin-eosin staining, x1000

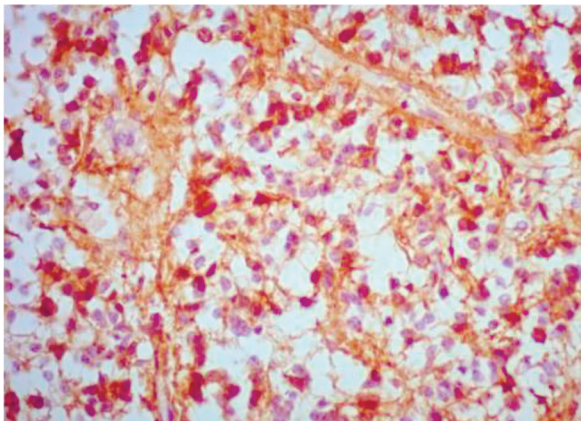

Figure 4. Moderate expression of MelanA in light pinealocytes of the gland of a child with extremely low body weight. Indirect peroxidase method. Reaction with MelanA, x1000

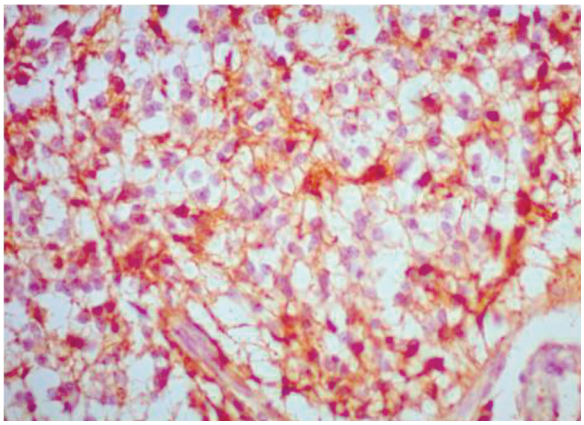

Figure 5. Moderate expression of S100 in light pinealocytes of the gland of a child with very low body weight. Indirect peroxidase method. Reaction with S100, x1000

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It is known that perinatal pathology in preterm infants with ELBW is accompanied by oxidative stress, which leads to free-radical damage of cells, tissues and organs [7]. The pineal gland produces the hormone melatonin. Melatonin (N-acetyl-5-methoxytryptamine), which is better known as a sleep regulator, performs many functions, revealing great versatility and diversity, as it has antioxidant, anti-inflammatory, anti-apoptotic and other properties [8-10]. The main metabolite of melatonin in urine is 6-sulfatoxymelatonin (6 - SM or aMT6s), which is a reliable surrogate biomarker reflecting the melatonin concentration in blood [11].

Our study found that the level of 6 - SM in the urine of preterm infants with ELBW in the first day of life has its own differences depending on the neonatal period. A significant decrease was determined in infants who had lethal outcomes. According to the literature, many perinatal conditions are characterized by oxidative stress, namely a decrease in the protective capacity of antioxidants against the background of hyperproduction of free radicals [12, 13]. Respiratory distress syndrome, hypoxic-ischemic encephalopathy, bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity, intracranial hemorrhage, neonatal sepsis and others are defined among perinatal diseases accompanied by oxidative stress.

It is known that immaturity and reduced adaptive mechanisms in preterm infants are depleted, starting as early as intrauterine, during birth and in the postnatal period of life, due to stress factors such as hypoxia, hyperoxia, reperfusion and inflammation [16]. For a deeper understanding of these processes, we performed a clinical and morphological comparison of the functional and structural features of the pineal gland of the brain in early infants with ELBW.

As a result of its small size, specific location, and multiple functional-anatomical connections with the intermediate brain and endocrine centers, the physiology of the pineal gland is poorly understood in both adults and children. Especially in preterm infants with ELBW. By applying macroscopic, histological, and histochemical methods, we have investigated some features of the structure and function of the pineal gland in infants with ELBW.

Considering that the infants we observed had a gestational age of 25 to 29 weeks, it can be argued that the pineal gland is already functioning during this period of intrauterine maturation. Its function increases with subsequent depletion. The obtained data on the increased morphofunctional activity of pineal cells in deceased infants confirm that this gland plays an extraordinary role in the processes of extrauterine adaptation and performs antioxidative protection. The results obtained open up the most important prospects for further research regarding drug supplementation of melatonin to preterm infants from the first day of life with full-scale center-based randomized trials.

Conclusions

1. Urinary excretion of the melatonin 6 metabolite sulfoxymelatonin in preterm infants with extremely low birth weight during the first day of life who had lethal outcomes was significantly reduced compared with surviving children (mean 65.5 pg/ml and 180.6 pg/ml, respectively), indicating in favor of depletion of the functional activity of the pineal gland in perinatal pathology.

2. Morphologically, there is an increase in morphofunctional activity of pineal cells in the brain epiphysis of preterm infants with extremely low birth weight, which is confirmed by morphometric data (pinealocyte area 93.4 μm2, nucleus area 38.7 μm2) and increased MelanA S100 expression in immunohistochemical study.

3. Chaotically arranged pinealocytes with foci of lysis in the periphery, areas of circulatory disorders, marginal chromatin in the nuclei and forced apoptosis are considered as evidence of compensation for perinatal pathology, which in turn leads to decreased synthesis of melatonin 6 and its mediated matabolite 6 - sulfoxymelatonin excreted with urine.

4. Based on the systematization of predictors of clinical and immunohistochemical compensatory effects of brain epiphysis in preterm infants with extremely low birth weight for diagnostic and prognostic nurture monitoring, it is reasonable to use urinary melatonin levels in the early neonatal period.

Prospects for future research. Based on their own published and literature data confirming the crucial role of the epiphysis in the neuroendocrine compensation of perinatal pathology, the authors consider it appropriate to direct future scientific clinical studies on the use of melatonin in preterm infants with determination of the effective dose, its duration of administration under control of pineal gland function.

The authors declare no conflict of interest.

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КЛІНІКО-МОРФОЛОГІЧНІ КОРЕЛЯТИ ФУНКЦІЇ ШИШКОПОДІБНОЇ ЗАЛОЗИ У НЕМОВЛЯТ З НАДЗВИЧАЙНО МАЛОЮ МАСОЮ ТІЛА ПРИ НАРОДЖЕННІ

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Резюме

Вступ. В адаптації до постнатального життя і у компенсації найбільш поширеної перинатальної патології передчасно народжених немовлят вкрай важлива роль належить функціональній активності шишкоподібної залози.

Мета дослідження. Визначити рівень 6-сульфоксімелатоніну сечі у першу добу життя у передчасно народжених немовлят з надзвичайно малою масою тіла та провести патоморфологічне дослідження зразків епіфізів у дітей, що померли, з подальшим клініко-морфологічним зіставленням.

Матеріал і методи дослідження. У 46 передчасно народжених дітей з надзвичайно малою масою тіла визначали рівень метаболіту мелатоніну в сечі 6-сульфоксімелатоніну у першу добу життя. У 20 померлих дітей проводили макро- й мікроскопічне дослідження шишкоподібної залози з використанням імуногістохімічного методу.

Результати дослідження. Усі передчасно народжені немовлята з надзвичайно малою масою тіла при народженні мали перинатальну патологію, яка призвела до летальних випадків у 20 з них. Екскреція з сечею метаболіта мелатоніну, 6-сульфоксімелатоніна, у передчасно народжених немовлят з надзвичайно малою масою тіла у першу добу життя, які мали летальні наслідки, достовірно зменшена у порівнянні з дітьми, які вижили, що свідчить на користь виснаження функціональної активності шишкоподібної залози та може слугувати маркером несприятливого перебігу неонатального періоду. Морфологічно в епіфізі мозку передчасно народжених немовлят з надзвичайно малою масою тіла відбувається посилення морфофункціональної активності пінеальних клітин. Це підтверджується морфометричними даними та збільшенням експресії MelanA і S100 при імуногістохімічному дослідженні.

Макро- та мікроскопічні дані свідчать про те, що позаутробне існування в умовах пеританальної патології обумовлює прискорення диференціювання і виснаження епіфізу з пошкодженням тканини залози, що призводить до зменшення синтезу мелатоніну та його опосередкованого метаболіта 6-сульфоксімелатоніну, який виділяється з сечею.

Висновки. Знижена екскреція 6-сульфоксімелатоніну із сечею у передчасно народжених немовлят з надзвичайно малою масою тіла в першу добу життя та мофологічні зміни в епіфізах дітей, що померли, свідчать про виснаження функціональної активності шишкоподібної залози при перинатальній патології.

Ключові слова: мелатонін; недоношеність; шишкоподібна залоза.

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