

CLINICAL STUDY

The role of vitamin D and trace elements in premature newborns with congenital pneumonia

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ABSTRACT

OBJECTIVES: The objective of the research is to determine the levels of vitamin D and trace elements in the umbilical cord blood, as well as to evaluate the clinical and laboratory characteristics in premature newborns with congenital pneumonia.

MATERIALS AND METHODS: This single-center case-control research included 228 premature newborns who were divided into the main group composed of 76 newborns with congenital pneumonia, and control group composed of 152 newborns without congenital pneumonia, who were born in the period from January 2021 to December 2021. An enzyme immunoassay of vitamin D determination was performed along with an assessment of clinical and laboratory characteristics. The modern mass spectrometry was performed to determine the trace element status of the blood of 46 premature newborns proven to have a severe vitamin D deficiency.

RESULTS: The results of our research demonstrated that premature newborns with congenital pneumonia had a severe vitamin D deficiency, low Apgar scores, and severe respiratory disorder (assessed by means of modified Downes score). The analysis showed that the newborns with congenital pneumonia had a significantly worse pH, lactate, HCO_3^- , and pCO_2 as compared with the newborns without pneumonia ($p < 0.05$). The analysis also revealed early biomarkers of congenital pneumonia in premature newborns, particularly thrombocytopenia, leukocytosis, high c-reactive protein (CRP) ($p < 0.05$).

Stage 2 of the research was performed with the use of modern mass spectrometry to determine the trace element status (in the first stage, vitamin D was determined) in 46 premature newborns proven to have a severe vitamin D deficiency. The examination revealed that the levels of iron, calcium, manganese, sodium, strontium were decreased, whereas the levels of magnesium, copper, zinc, aluminum, and arsenic were increased. Only potassium, chromium, and lead turned out to be in normal range. According to the available data, in contrast to the most of micronutrients in the context of inflammatory response, the plasma concentrations of copper and zinc are reported to be increased as opposed to iron, the concentration of which is shown to be decreased.

CONCLUSION: Our results demonstrated a high prevalence of 25 (OH) vitamin D deficiency in premature newborns. A significant relationship has been shown between the respiratory status of vitamin D and presence of congenital pneumonia in premature newborns. The analysis also showed that the content of trace elements in premature newborns plays an immunomodulatory role and affects the susceptibility and outcome of the infectious process. Thrombocytopenia in premature newborns may be an early biomarker for monitoring congenital pneumonia (Tab. 2, Ref. 28). Text in PDF www.elis.sk

KEYWORDS: congenital pneumonia, premature newborn, vitamin D, trace elements, mass spectrometry.

Introduction

Congenital pneumonia, in the modern world today, remains an important and urgent problem. The incidence rate of this pathology varies around 1% among the mature newborns, while in premature newborns, it reaches up to 10%. As to neonatal mortality, it occupies a leading place with a rate of 10 to 40% of cases (1). Nissen MD noted that pneumonia was the cause of about 1.2 mil-

lion neonatal deaths per year, which in turn accounted for 10% of infant deaths worldwide (2).

According to a systematic analysis of the research of the global burden of diseases, congenital pneumonia remains a complex pathological process encountered especially in the developing countries but also in modern healthcare (3).

The immune system plays a major role in protecting the body against infections (4). The innate immune system includes external protection, phagocyte cells, cytokine groups, interferons and serum proteins, which provide an important role in protecting against infection (3, 5). Trace elements and vitamins, together referred to as micronutrients, are essential for metabolism and activation of the immune system of the human body.

Vitamin D is a fat-soluble hormone which is formed predominantly under the influence of ultraviolet rays and sunlight in the hu-

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man skin and to a lesser extent, it comes from food, and then enters the liver with the help of a vitamin D-binding protein. In the liver, vitamin D is converted into 25-hydroxyvitamin D (25(OH)D), the levels of which are examined to determine the status of vitamin D (6). The physiological effects of vitamin D are very important because it plays a role in mineral homeostasis and bone function, including the regulation of immunity, fetal development and lung function (7, 8, 9). The positive effect of steroids on the lung development is well known and 1.25-dihydroxyvitamin D₃ has been shown to have a positive effect on the fetal lung development. The 25-hydroxyvitamin D (25(OH)D) is an important steroid hormone involved in the synthesis of surfactant, which plays a role in the cell development and promotes the maturation of pulmonary system (10, 11).

Minerals and vitamins are cofactors and activators of the developing immune system in newborns. According to research results, it is known that congenital nutritional deficiency is associated with a reduced thymus function. The antigenic status of a newborn depends on the state of the fetal neuroendocrine system, neonatal microbial flora and maternal factors. In the postnatal period, minerals and vitamins are involved in triggering the body's immune response by influencing the innate mechanisms of immune signal transmission and immune cell development. Insufficient vitamin D level can impair the immune response and therefore affects the integrity of the intestine and immune function of the mucous membrane in the newborns (12). Vitamin D and trace elements, particularly magnesium, copper, zinc and iron play an important role in maintaining the immune system (13). Micronutrients are also involved in maintaining the activity of antimicrobial proteins. It is known that an imbalance in the nutritional status increases the risk and severity of infection, and therefore it is necessary to maintain a sufficient level of each trace element (14).

Objective. The objective of the research is to determine the levels of vitamin D and trace elements in the umbilical cord blood, as well as to evaluate the clinical and laboratory characteristics in premature newborns with congenital pneumonia.

Materials and methods of research

Research design: case control.

The research was conducted at the Regional Perinatal Center in Semey city, Republic of Kazakhstan in the period from January 01, 2021, to December 31, 2021. The research included 228 premature newborns. The research was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the local ethics committee. All participants (mothers) signed a voluntary informed consent. The mothers were informed about the processing of the obtained data, and on the fact that the findings are published without disclosing any personal data.

The first stage of the research lied in the determination of vitamin D levels in 228 premature newborns along with evaluation of their clinical and laboratory characteristics. Stage 2 of the research was aimed at determining trace elements in the blood of 46 premature newborns who had been identified as having a severe vitamin D deficiency. The latter examination was performed by use of modern mass spectrometry.

Immediately after birth, umbilical cord blood samples were taken into a vacutainer without filler in a volume of 5.0 ml (EVACUATED TUBE Single-use Blood Collection System). Then this test tube was placed in a centrifuge (Sky Line Centrifuge CM-6M, with parameters of 3,000 revolutions) for 3 minutes. The finished serum was taken using a 1.0 ml dispenser and then placed in an Eppendorf 1-ml test tube subjected to a temperature of -20°C and transported in a cold bag with ice packs to the Center of the Research Laboratory of the Semey Medical University in Semey city, Republic of Kazakhstan. An enzyme immunoassay was performed to quantify 25OH-D3 using a set of 25-OH Vitamin D total ELISA (96 samples). Demeditec 25-OH Vitamin D total ELISA is a solid-phase immunosorbent enzyme assay performed on a microplate. Within the first 2 hours of incubation at room temperature, the total 25-OH vitamin D (D2 and D3) present in the calibrators, controls and samples is separated from the serum-binding proteins to bind with the binding sites of a specific monoclonal antibody. After the 1st wash, a certain amount of 25-OH vitamin D marked with biotin in the presence of horseradish peroxidase (HRP), along with unmarked 25-OH vitamin D2 and 25-OH vitamin D3, is present on the binding sites of a specific monoclonal antibody. After 30 minutes of incubation at room temperature, the microplate is rinsed with water to stop the competing reaction. A chromogenic solution (TMB) is added, and then incubated for 15 minutes. The reaction stops when a stopping solution is added, and then the microtiter plate is read at the appropriate wavelength. The amount of the substrate volume is determined colorimetrically by changing the absorption coefficient, which is inversely proportional to the concentration of total 25-OH vitamin D (D2 and D3). A calibration curve is constructed, and the concentrations of total 25-OH vitamin D (D2 and D3) in the samples are determined by dose interpolation from the calibration curve. In this research, AIFR-01 UNIPLAN analyzer was used.

The umbilical cord blood was also collected in a vacutainer without filler in a volume of 1.0 ml (EVACUATED TUBE Single-use Blood Collection System); the biological material was immediately placed in a freezer at a temperature T of -20°C . The determination of the content of chemical elements was carried out by ICP-MS methods, using an iCAP Q quadrupole mass spectrometer from Thermo Scientific.

Blood gas test was taken at the time of birth, and a general blood test and biochemical blood test (c-reactive protein; CRP) were taken in the first 8–12 hours of life.

Inclusion criterion for gestational age was in range of 22 to 37 weeks. Exclusion criteria were as follows: children with congenital malformation, genetic diseases, and mature newborns.

The main group was composed of premature newborns with congenital pneumonia (76 newborns).

The control group was composed of premature newborns without congenital pneumonia (152 newborns).

Statistical analysis

Statistical data processing was carried out using SPSS software 20.0. Quantitative data were evaluated for their compliance with normal distribution using the Kolmogorov–Smirnov criterion.

Quantitative indicators with normal distribution were described using the arithmetic mean (M) and standard deviations (SD), limits of 95% confidence interval (95% CI); for comparison, a Student's test for independent samples was used. Categorical data were described using absolute values and percentages, while Pearson's χ^2 , continuity correction, and Fisher's criterion were used to identify the relationships between nominal variables. The differences between the compared variables were considered significant at $p < 0.05$.

Results

General characteristics

This research included 228 newborns while the main group and control group included 76 (33.3 %) and 152 (66.7 %) children, respectively. At the same time, in the main group, 44 children (57.9 %) were male, and 32 children (42.1 %) were female, while in the control group, 67 children (44.1 %) were male and 85 children (55.9 %) were female.

The mean body weight of children at birth was 1761.6 g (95% CI: 1687.3–1835.9) with SD = 569.3; the minimum and maximum body weights were 470 and 3,000 grams, respectively. The mean body weight of children in the main group at birth was 1,461.3 grams (95% CI: 1337.3–1585.3) with SD = 542.7; the minimum and maximum body weights of children at birth in this group were 470 and 3,000 grams, respectively. The mean weight of children in the control group was 1,911.8 grams (95% CI: 1828.1–1995.5) with SD = 522.4; the minimum and maximum body weights of children at birth in this group were 690 and 3,000 grams, respectively.

The mean Apgar score in the 1st minute was 5.48 (95% CI: 5.26–5.71) with SD = 1.71; the minimum and maximum scores were equal to 1 and 8, respectively. In the main group, the mean Apgar score in the 1st minute was 4.39 (95% CI: 3.96–4.83) with SD = 1.89; the minimum and maximum scores were equal to 1 and 7, respectively. In the control group, the mean Apgar score in the 1st minute was 6.03 (95% CI: 5.82–6.24) with SD = 1.32; the minimum and maximum scores were equal to 2 and 8, respectively. The quantitatively continuous type of the data, presence of two independent samples, normal distribution of the trait, and equality of dispersion ($p = 0.223$) allowed the Student's test to be applied to compare the mean Apgar scores in the 1st minute. The mean Apgar score in the 1st minute in the main group was lower than in the control group by 1.63 (SD = 0.215; $t = 7.583$; $df = 226$; $p = 0.000$); the differences are statistically significant.

The mean Apgar score in the 5th minute was equal to 6.37 (95% CI: 6.18–6.56) with SD = 1.46; the minimum and maximum scores were equal to 2 and 9, respectively. In the main group, the mean Apgar score in the 5th minute was equal to 5.33 (95% CI: 4.95–5.71) with SD = 1.68; the minimum and maximum scores were equal to 2 and 8, respectively. In the control group, the mean Apgar score in the 5th minute was equal to 6.89 (95% CI: 6.73–7.05) with SD = 1.00; the minimum and maximum scores were equal to 3 and 9, respectively. The quantitatively continuous type of the data, presence of two independent samples, normal distribution of the trait, and equality of dispersion ($p = 0.342$) allowed the Student's test to be applied to compare the mean Apgar score in the 5th minute.

The mean Apgar score in the 5th minute in the main group was lower than in the control group by 1.56 (SD = 0.177; $t = 8.8203$; $df = 226$; $p = 0.000$); the differences are statistically significant.

For early detection and evaluation of the severity of respiratory disorders in newborns, we used a modified Downes score (15). The mean modified Downes score was equal to 5.00 (95% CI: 4.62–5.37) with SD = 2.84; the minimum and maximum scores were equal to 0 and 10, respectively. In the main group, the mean modified Downes score was equal to 7.32 (95% CI: 6.93–7.71) with SD = 1.71; the minimum and maximum scores were equal to 4 and 10, respectively. In the control group, the mean modified Downes score was equal to 3.84 (95% CI: 3.42–4.25) with SD = 2.57; the minimum and maximum scores were equal to 0 and 9, respectively. The quantitatively continuous type of data, presence of two independent samples, normal distribution of the trait, and equality of dispersion ($p = 0.211$) allowed the Student's test to be applied to compare the mean modified Downes score. The mean modified Downes score in the main group was higher than in the control group by 3.48 (SD = 0.177; $t = 10.764$; $df = 226$; $p = 0.000$); the differences are statistically significant.

The vitamin D content was evaluated based on the data from Holick MF, Binkley NC, with following criteria for 25 (OH)D concentration (16):

- Norm in range of 30–80 ng/ml;
- Insufficiency in range of 20–30 ng/ml,
- Deficiency in range of 10–19 ng/ml,
- Severe deficiency less than 10 ng/ml.

The mean level of 25(OH)D was equal to 18.0 ng/ml (95% CI: 17.0–19.0) with SD = 7.77; the minimum and maximum levels were equal to 1 and 32 ng/ml, respectively. At the same time, in the main group, the mean level of 25(OH)D was equal to 12.0 ng/ml (95% CI: 10.3–13.6) with SD = 7.28; the minimum and maximum levels were equal to 1 and 30 ng/ml, respectively. In the control group, the mean level of 25(OH)D was equal to 21.0 ng/ml (95% CI: 20.1–22.0) with SD = 6.07; the minimum and maximum levels were equal to 4 and 32 ng/ml, respectively.

A severe deficiency of 25(OH)D was found to be present in 43 children (18.9 %), deficiency in 80 children (35.1 %), insufficiency in 96 newborns (42.1 %), and normal level in 9 newborns (3.9 %). In the main group, a severe deficiency of 25(OH)D was found to be present in 39 children (51.3 %), deficiency in 21 children (27.6 %), insufficiency in 15 newborns (19.7 %), and normal level in 1 newborn (1.3 %). In the control group, a severe deficiency of 25(OH)D was found to be present in 4 children (2.6 %), deficiency in 59 children (38.8 %), insufficiency in 81 newborns (53.3 %), and normal levels in 8 newborns (5.3 %); ($\chi^2 = 81.028$; $df = 3$; $p = 0.000$).

The level of trace elements was determined in 46 children with a severe vitamin D deficiency. The data are presented in Table 1.

As can be seen from Table 1, the calcium level in most of the children of this group was decreased. The levels of magnesium and copper in most of children were increased. The level of zinc was mainly increased; only 34.8 % of children had this indicator in normal range. The potassium level was mostly normal (69.6 %). The level of aluminum in almost all children (97.8 %) was

Tab. 1. Trace elements. Mass spectrometry.

Trace elements	Results					
	Mean (95% CI)	SD	Min. Max	Decreased	Norm	Increased
Ca	74.6 (62.3–86.9)	41.4	24; 235	28 (60.9%)	10 (21.7%)	8 (17.4%)
Mg	50.1 (46.4–53.8)	12.3	18; 74	0 (0%)	1 (2.2%)	45 (97.8%)
Cu	1.0 (0.7–1.3)	1.0	0; 6	9 (19.6%)	0 (0%)	37 (80.4%)
Zn	3.8 (3.0–4.7)	2.8	0; 10	1 (2.2%)	16 (34.8%)	29 (63.0%)
K	2100.6 (1945.2–2255.9)	523.2	29; 2889	1 (2.2%)	32 (69.6%)	13 (28.3%)
Al	36.0 (29.9–42.0)	20.3	0; 107	–	1 (2.2%)	45 (97.8%)
Cr	0.6 (0.3–0.9)	1.1	0; 3	–	35 (76.1%)	11 (23.9%)
Fe	495.8 (466.8–524.8)	97.6	167; 668	46 (100.0%)	0 (0%)	0 (0%)
Pb	0.02 (–0.02–0.07)	0.15	0; 1	–	45 (97.8%)	1 (2.2%)
Mn	0.5 (–1.1–2.1)	0.54	0; 2	35 (76.1%)	0 (0%)	11 (23.9%)
Na	1740.0 (1606.4–1873.7)	450.1	1070; 2675	46 (100.0%)	0 (0%)	0 (0%)
As	0.10 (0.01–0.19)	0.303	0; 1	–	41 (89.1%)	5 (10.9%)
Sr	0.07 (–0.03–0.16)	0.33	0; 2	44 (95.7%)	0 (0%)	2 (4.3%)

Tab. 2. Acid-base state and blood test.

	Main group			Control group			χ^2 , df, p
	Decreased	Norm	Increased	Decreased	Norm	Increased	
pH	76 (100.0%)	0 (0.0%)	0 (0.0%)	144 (94.7%)	8 (5.3%)	0 (0.0%)	$\chi^2=4.145$, df=1, p=0.042
Lactate	2 (2.6%)	11 (14.5%)	63 (82.9%)	51 (33.6%)	82 (53.9%)	19 (12.5%)	$\chi^2=110.00$, df=2, p=0.000
HCO ₃	24 (31.6%)	47 (61.8%)	5 (6.6%)	18 (11.8%)	126 (82.9%)	8 (5.3%)	$\chi^2=13.83$, df=2, p=0.001
pCO ₂	3 (3.9%)	6 (7.9%)	67 (88.2%)	0 (0.0%)	29 (19.1%)	123 (80.9%)	$\chi^2=10.45$, df=2, p=0.005
WBC	0 (0.0%)	72 (94.7%)	4 (5.3%)	0 (0.0%)	152 (100.0%)	0 (0.0%)	$\chi^2=8.14$, df=1, p=0.004
PLT	32 (42.1%)	44 (57.9%)	0 (0.0%)	20 (13.2%)	132 (86.8%)	0 (0.0%)	$\chi^2=24.12$, df=1, p=0.000
CRP	–	35 (46.1%)	41 (53.9%)	–	149 (98.0%)	3 (2.0%)	$\chi^2=87.88$, df=1, p=0.000

increased. The chromium level in most of children (76.1 %) was normal. In all children of the main group, the iron level was decreased. The lead level in almost all children in this group was normal. The level of manganese was mainly decreased (76.1 %). There were no normal indicators of manganese levels in any child, and almost a quarter of children (23.9 %) had this indicator increased. The sodium level in all children in the main group was decreased. The level of arsenic was within the permissible norm in the majority of children (89.1 %); a small number of children had increased levels of arsenic (10.9 %). The level of strontium was decreased in almost all children of the main group (95.7 %); in 4.3 % of children of this group it was increased.

Acid-base physiology and interpretation of blood gases were performed in all newborns included in the study (228) and compared between the main and control groups. The analysis showed that the newborns with congenital pneumonia had worse pH, lactate, HCO₃ and pCO₂ as compared with the newborns without pneumonia; the difference is statistically significant ($p < 0.05$). The data are presented in Table 2 (Acid-base state and blood test). The reference indicators of the acid-base state of the blood correspond to those given by the authors (17, 18).

In the general blood analysis, thrombocytopenia and leukocytosis were noted to be present in the main group ($p < 0.05$). Also, in the biochemical analysis, the level of c-reactive protein (CRP) was significantly higher compared to the control group; the laboratory data had statistically significant differences.

Discussion

The research demonstrated that the level of vitamin D in the blood serum of premature newborns with congenital pneumonia was significantly lower compared to the control group. The mean vitamin D level in premature newborns with congenital pneumonia was equal to **12.0 ng/ml** (95% CI: 10.3–13.6) with SD = 7.28 and minimum and maximum levels were equal to 1 and 30 ng/ml, respectively, whereas in the premature newborns without pneumonia, the mean level of 25(OH)D was equal to **21.0 ng/ml** (95% CI: 20.1–22.0) with SD = 6.07 and minimum and maximum levels were equal to 4 and 32 ng/ml, respectively.

A similar finding was reported by Gad Ghada I, Abushady Nancy M, Fathi Marwa S and others, as well as by Gallo RL, and Murakami M, who showed that the low vitamin D content is characteristic in response to a number of specific infections (19, 20).

The general characteristics of the patients who took part in our research corresponded to those in previously conducted large-scale research studies, including the randomized ones. In our research, 57.9 % and 44.1 % of newborns in the main and control groups were male, respectively. These findings are confirmed in other previous studies (21). The mean body weight in the children with congenital pneumonia was 1,461.3 grams (95% CI: 1337.3–1585.3) with SD = 542.7, while in children without congenital pneumonia, it was 1,911.8 grams (95% CI: 1828.1–1995.5) with SD = 522.4.

The results of our research are comparable to those published by Onwuneme Ch and co-authors, as well as by Belderbos ME and co-authors who describe similar data (21, 22, 23). In this research, we found that the premature newborns with congenital pneumonia had their Apgar scores at 1st and 5th minutes lower than the premature newborns without congenital pneumonia. ($p < 0.05$).

For early detection and evaluation of the severity of respiratory disorders in newborns, we used a modified Downes score (15). The mean modified Downes score was equal to 5.00 (95% CI: 4.62–5.37) with SD = 2.84 and minimum and maximum scores equal to 0 and 10, respectively. In the main group, the mean modified Downes score was equal to 7.32 (95% CI: 6.93–7.71) with SD = 1.71 and minimum and maximum scores equal to 4 and 10, respectively. In the control group, the mean modified Downes score was equal to 3.84 (95% CI: 3.42–4.25) with SD = 2.57 and minimum and maximum scores equal to 0 and 9, respectively. The quantitatively continuous type of data, presence of two independent samples, normal distribution of the trait, and equality of dispersion ($p = 0.211$) allowed the Student's test to be applied in the comparison of mean modified Downes scores. The mean modified Downes score in the main group was higher than that in the control group by 3.48 (SD = 0.177; $t = 10.764$; $df = 226$; $p = 0.000$); the differences are statistically significant.

In their research, Balan KV and co-authors as well as we in our research, noted a high prevalence of children with a low level of 25(OH)D and established the relationship of vitamin D status with acute respiratory pathology in premature newborns after birth (24).

Our research encompasses also the factor of trace elements status in the premature newborns with congenital pneumonia concurrent with severe deficiency in 25(OH)D. According to the results of our research, the levels of iron, calcium, manganese, sodium, and strontium were decreased, whereas the levels of magnesium, copper, zinc, aluminum, and arsenic were increased. Only potassium, chromium, and lead turned out to be in normal range. According to the available data, unlike most of micronutrients in the context of an inflammatory reaction, the concentration of copper and zinc in plasma increases, while the concentration of iron decreases, which is similar to our results (25).

The research also showed that children with congenital pneumonia had thrombocytopenia and leukocytosis ($p < 0.05$). Also the biochemical analysis showed that the level of c-reactive protein (CRP) was significantly higher compared to the control group. These results are comparable with the data of other authors (26). Thrombocytopenia is known as an early and nonspecific serum marker of inflammation and sepsis (27, 28).

Conclusions

The results of our research demonstrated a high prevalence of 25(OH) vitamin D deficiency in the premature newborns. Our study has shown the relationship between the respiratory status of vitamin D and presence of congenital pneumonia in the premature newborns to be significant. The analysis also showed that the content of trace elements in premature newborns plays an im-

munomodulatory role and affects the susceptibility and outcome of the infectious process. Thrombocytopenia in the premature newborns may be an early biomarker for monitoring congenital pneumonia.

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