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LEVEL OF VITAMIN D AND CATHELICIDIN IN PRETERM INFANTS WITH CONGENITAL PNEUMONIA

N. E. Verisokina [1, 2], L. Ya. Klimov [1], M. A. Petrosyan [1, 2], V. V. Zubkov [3], T. V. Zheleznyakova [1, 2], A. Alkhimidi [1, 4], N. V. Zarytovskaya [1], A. L. Zaplatnikov [5]

[1] Stavropol State Medical University, Russian Federation
[2] Stavropol Regional Clinical Perinatal Center, Russian Federation
[3] National Medical Research Center of Obstetrics, Gynecology and Perinatology named after Academician V. I. Kulakov, Moscow, Russian Federation
[4] General Hospital Al-Wajh, Saudi Arabia
[5] Russian Medical Academy of Continuing Professional Education, Moscow, Russian Federation

УРОВЕНЬ ВИТАМИНА D И ПРОДУКЦИЯ КАТЕЛИЦИДИНА У НЕДОНОШЕННЫХ НОВОРОЖДЕННЫХ С ВРОЖДЕННОЙ ПНЕВМОНИЕЙ

Н. Е. Верисокина [1, 2], Л. Я. Климов [1], М. А. Петросян [1, 2], В. В. Зубков [3], Т. В. Железнякова [1, 2], А. Альхимиди [1, 4], Н. В. Зарытовская [1], А. Л. Заплатников [5]

[1] Ставропольский государственный медицинский университет, Российская Федерация
[2] Ставропольский краевой клинический перинатальный центр, Российская Федерация
[3] Национальный медицинский исследовательский центр акушерства, гинекологии и перинатологии им. академика В. И. Кулакова, Москва, Российская Федерация
[4] Больница общего профиля Аль-Ваджа, Саудовская Аравия
[5] Российская медицинская академия непрерывного профессионального образования, Москва, Российская Федерация

The level of antimicrobial peptides, which affects the course of the infectious process, depends on the concentration of vitamin D in the blood serum. The analysis of anthropometric and laboratory data in 90 premature newborns was carried out in the Stavropol Regional Clinical Perinatal Center and Stavropol Regional Clinical Perinatal Center № 1. Calcidiol medina in the study group was 17.9 [12.2; 23.3] ng/ml. At the same time, 54 (60 %) infants had 25(OH)D deficiency.

Premature newborns who did not have an infectious process had a statistically significantly higher median calcidiol than infants who had congenital pneumonia and died (20.1 [16.2; 33.0] ng/ml, 17.9 [10.8; 23.4] ng/ml ($p<0.05$) and 13.8 [10.1; 17.1] ng/ml ($p<0.01$), respectively). The cathelicidin level in the group of children with congenital pneumonia was 1.1 [0.5; 2.4] ng/ml, without it – 1.1 [0.6; 2.1] ng/ml, which is almost two times lower than in the group of deceased newborns. The study found that a normalized level of 1.25-dihydroxycholecalciferol allows adequate synthesis of cathelicidin, leading to a reduced risk of adverse outcomes from infectious processes in premature newborns.

Keywords: premature newborn, vitamin D, cathelicidin, congenital pneumonia

Известно, что от концентрации витамина D в сыворотке крови зависит уровень антимикробных пептидов, который влияет на течение инфекционного процесса. В Ставропольском краевом клиническом перинатальном центре и Ставропольском краевом клиническом перинатальном центре № 1 был проведен анализ антропометрических и лабораторных данных 90 недоношенных новорожденных. Медиана кальцидиола в исследуемой группе – 17,9 [12,2; 23,3] нг/мл. При этом 54 (60 %) младенца имели дефицит 25(OH)D.

Недоношенные новорождённые без инфекционного процесса имели статистически значимо более высокую медиану кальцидиола, чем младенцы с врожденной пневмонией и умершие – 20,1 [16,2; 33,0] нг/мл, 17,9 [10,8; 23,4] нг/мл ($p<0,05$) и 13,8 [10,1; 17,1] нг/мл ($p<0,01$) соответственно. Уровень кателицидина в группе детей с врожденной пневмонией составил 1.1 [0,5; 2,4] нг/мл, без нее – 1,1 [0,6; 2,1] нг/мл, что ниже практически в 2 раза, чем в группе умерших новорожденных. Таким образом, поддержание концентрации 1,25-дигидроксихолекальциферола в пределах физиологической нормы позволяет синтезировать LL-37, что снижает риск развития неблагоприятных исходов при инфекционном процессе.

Ключевые слова: недоношенный новорожденный, витамин D, кателицидин, врожденная пневмония

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CD3+CD19 – T-lymphocytes
ELISA – enzyme immunoassay
LL-37 – cathelicidin
RXR – retinoid X-receptors
VDR – vitamin D receptor
1.25(OH)2D – calcitriol
25(HE)D – calcidiol

Many studies indicate that 25(OH)D, starting from the period of human intrauterine development, takes part in regulating the functions of various organs and systems [1, 2]. Vitamin D is an essential prehormone involved in many metabolic processes, possessing endocrine, exocrine, and paracrine multisystem effects, and calcidiol deficiency is recognized as a pandemic with many negative health consequences [3–5]. In 50–90 % of adults and children in Russia, low vitamin D and children's vitamin D concentrations have been registered, as evidenced by studies [6].

The fetus cannot synthesize its own 25(OH)D. Thus, the vitamin D concentration in the newborn depends on its level in the mother's serum. The mother's calcidiol is transferred to the fetus through the placenta. After that, 1.25-dihydroxycholecalciferol is synthesized using the enzyme 1-α-hydroxylase. It should be noted that the metabolism and transmission of vitamin D and its effect on the placenta have not been thoroughly studied [7–9].

Hormonal relationships in the «mother – placenta – fetus» system consist not only of metabolism, nutrition, and barrier protection, from the point of view of embryonic development but also of providing biologically active substances, which include 25(OH)D and its metabolites [10]. In addition, the establishment of calcidiol depot during the prenatal period provides several functions essential for growth and development, including regulating cell differentiation and apoptosis, immune system, and brain development in the child [11]. Cathelicidins belonging to the group of antimicrobial peptides express bactericidal activity against bacteria and fungi [12]. LL-37 consists of 37 amino acid residues [13]. The presence of bacteria, viruses, and fungi contributes to synthesizing cathelicidin, which lyzes the infectious pathogen [14].

Generalization of scientific data proves that calcidiol levels affect the concentration of antimicrobial proteins that influence the course of the infectious process [15]. For example, an association was found between insufficient concentration of 25(OH)D and the development of respiratory tract infections in newborns. Sufficient synthesis of 1.25-dihydroxyvitamin D3 increases the production of cathelicidin. With calcidiol deficiency (<20 ng/ml), the production of LL-37 is reduced [16]. In general scientific availability, there are few studies on the effect of calcidiol intake during pregnancy on the synthesis of LL-37.

Our study aims to analyze the concentration of calcidiol and antimicrobial peptide cathelicidin in the serum of premature neonates with and without congenital pneumonia.

Material and Methods. From February 2018 to January 2020, in the Stavropol Regional Clinical Perinatal Center and Stavropol Regional Clinical Perinatal Center № 1, 90 premature newborns were examined, and anthropometric and laboratory data were analyzed. There were 40 girls (44.4 %) and 50 boys (55.6 %). Fifty two (57.8 %) children were born through cesarean section operation, 38 (42.2 %) infants were born through the natural birth canal. The gestational age of premature newborns was 31.0 [29.0–33.0] a week. The weight of infants at birth was 1490.0 [1100.0; 1900.0] grams. Height – 41.0 [36.0; 45.0] cm.

At the same time, 56 (62.3 %) premature newborns had pneumonia that occurred in the first 72 hours of life, 24 (26.8 %) did not have this pathology, 7 (7.7 %) newborns died in the first week of life, 3 (3.2 %) infants from the 8th to the 28th day of life.

The ELISA method was used to determine the content of calcidiol and LL-37 in blood serum, which was sampled in the first 72 hours of life. The serum calcidiol index <10 ng/ml corresponds to a severe deficiency of 25 (OH)D; from 10 to 20 ng/ml – deficiency; from 20 to 30 ng/ml – insufficiency; the optimal level is >30 ng/ml [17]. There are no established criteria for evaluating the cathelicidin index.

Using the add-in to Excel AtteStat (Analytera, Russian Federation), we conducted a statistical assessment and analysis of the data. Shapiro – Wilk test was used to test normality. The median (Me), 1st and 3rd quartiles were determined for each indicator in the studied groups [25Q; 75Q]. The Mann – Whitney U-test was used to compare numerical indicators in different groups. The Pearson pair correlation coefficient (r) was used to estimate the linear relationship between the indicators. The difference was considered statistically significant at $p<0.05$.

Results and Discussion. Preterm newborns were born at a tertiary care hospital to high-risk mothers. In the group of children without congenital pneumonia, ventilation was required in 12.5 % of cases, whereas children with congenital pneumonia and deceased infants in 64.3 % and 100 % of cases, respectively.

The median calcidiol level in the study group was 17.9 [12.2; 23.3] ng/ml. Calcidiol levels <10 ng/ml were in 12 (13.3 %) premature newborns, 10–20 ng/ml – 42 (46.7 %), 20–30 ng/ml – 20 (22.2 %) children, and only 16 (17.8 %) premature newborns had a concentration of 25(OH)D > 30 ng/ml. The levels of calcidiol and LL-37 in premature newborns of the studied groups are shown in Figure 1.

After analyzing laboratory parameters, it can be concluded that the vitamin D concentration in the blood serum of premature newborns with an infectious process was statistically significantly lower than in children without it ($p<0.05$). The concentration of calcidiol in the blood serum of deceased infants was 1.3 times lower than in newborns born prematurely with pneumonia ($p>0.05$). It was detected in the first 72 hours of life and was 1.5 times lower than in children without it ($p<0.01$).

In newborns born prematurely, who do not have an infectious process, and infants with pneumonia detected

in the first three days after birth, the LL-37 index did not differ. The LL-37 index was almost two times lower in deceased infants than in premature newborns with congenital pneumonia and children without it (p>0.05) (Fig. 2).

A statistically significantly positive relationship was found between the level of calcidiol and cathelicidin in deceased premature newborns (r=0.71, p<0.05).

As the results of recent studies show, vitamin D affects many body functions. Calciferol deficiency in pregnant women and young children increases the risk of non-infectious and infectious pathologies [18]. Role 25(OH)D during pregnancy, perinatal outcomes and the formation of a child's health is of considerable importance [19]. Calcitriol is involved in the regulation of phosphorus-calcium metabolism and affects the work of immune cells. Vitamin D receptor (VDR) discovered in immune cells (on unformed lymphocytes of the thymus and mature CD8 cells, CD3+CD19+, macrophages) served as confirmation of the role of calcidiol in the immune system. 25(OH)D protects against infectious pathology by modulating innate and adaptive immunity. For the first time, the relationship between infection and 25(OH)D was written more than 150 years ago [7, 13].

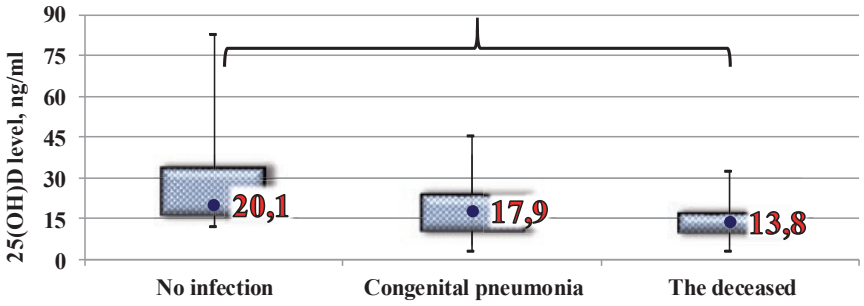

Fig. 1. Calcidiol levels in premature newborns in different groups

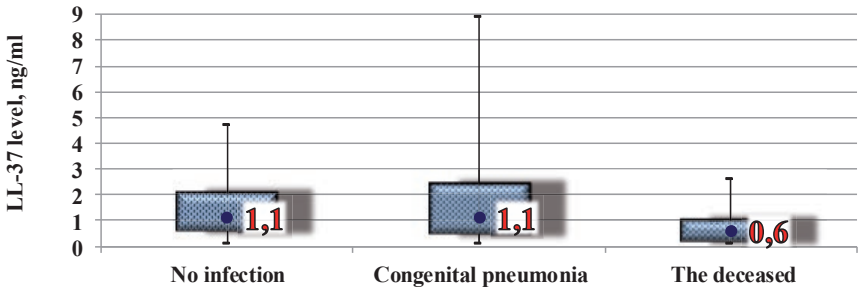

Fig. 2. The level of cathelicidin in premature newborns in different groups

Calcitriol is the active form of vitamin D3 and increases phagocytic function and chemotaxis of immune cells. Bacteria, viruses, and fungi are recognized by Toll-like receptors that enhance the expression of VDR and 1α-hydroxylase. After transport to cell 25(OH)D is metabolized into the active form – 1.25-dihydroxyvitamin D. 1.25(OH)2D3 forms a compound with VDR and RXR, which activates the synthesis of many antimicrobial peptides and proteins, for example, β-defensin-2 and LL-37 [13, 14, 20]. 1.25-dihydroxyvitamin D3 binding to VDR increases the expression of LL-37 at the gene level. Cathelicidin molecules realize their antibacterial abilities in phagosomes [14]. The 1.25(OH)2D produced in macrophages objectively causes paracrine effects, acting on the function of T- and B-lymphocytes and monocytes. In addition to the signaling of Toll-like receptors, the expression of 1α-hydroxylase is influenced by other cytokines, such as interferon-γ or interleukin-4 [18].

In countries where a large proportion of the population is vitamin D deficient, several studies have linked low calcidiol levels to the risk of respiratory and other infectious diseases. For example, a study conducted in Turkey involving 30 full-term newborns who were admitted to the intensive care unit with respiratory tract infection after birth and 30 healthy ones showed that the average level of 25(OH)D in patients in the study group was 29 nmol/l (20–31 nmol/l) compared with 43 nmol/l (34–52 nmol/l) for the control group [21].

On the other hand, vitamin D deficiency increases the risk of sepsis. Calcidiol may reduce the risk of sepsis by increasing the level of cathelicidin. Scientific evidence in the literature studies the relationship between the concentration of 25(OH)D in mothers and newborns and the risk of sepsis in newborns [22, 23].

Quite comparable to the data of foreign colleagues, for example, from 2015 to 2016, a study on the effect of calcidiol deficiency as a possible risk factor for late-onset sepsis in premature and preterm newborns was conducted in northern India. The average vitamin D level in the main group was significantly lower than in the control group – 15.4 ng/ml and 21.4 ng/ml (p=0.001), respectively. Women who gave birth to children with late sepsis also had significantly lower vitamin D levels than mothers of children from the control group – 17.9 ng/ml and 23.7 ng/ml (p=0.004), respectively [24, 25].

Conclusion. The analysis of laboratory data allows us to conclude that there is a 25(OH)D deficiency in 60 % of newborns born prematurely. Newborns born prematurely, without an infectious process, had a higher median of 25(OH)D – 20.1 [16.2; 33.0] ng/ml than infants with pneumonia detected in the first 72 hours of life – 17.9 [10.8; 23.4] ng/ml (p<0.05) and deceased – 13.8 [10.1; 17.1] ng/ml (p<0.01). The cathelicidin index of deceased newborns was significantly lower than in infants with pneumonia that occurred in the prenatal period and manifested itself in the first three days of life after birth.

The immunotropic effect of vitamin D realized through the production of antimicrobial peptides, affects the prognosis of infectious processes in newborns. Vitamin D deficiency, correlated with decreased LL-37 levels, is an unfavorable factor that increases the risk of death in congenital pneumonia.

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About authors:

Verisokina Natalia Evgenievna, CMSc, Associate Professor of the Department of Faculty Pediatrics, neonatologist;
tel.: +79624542117; e-mail: veris.natalya@yandex.ru; https://orcid.org/0000-0001-5444-8351

Klimov Leonid Yakovlevich, MD, DMSc, Professor, Head of the Department;
tel.: +79289630261; e-mail: klimov_leo@mail.ru; https://orcid.org/0000-0001-7248-1614

Petrosyan Meline Arturovna, Assistant of the Department of Faculty Pediatrics, neonatologist;
tel.: +79682667639; e-mail: meline.petrosyan.94@bk.ru; https://orcid.org/0000-0001-9906-7501

Zubkov Viktor Vasilyevich, MD, DMSc, Professor, Director of the Institute of Neonatology and Pediatrics,
Head of the Department of Neonatology; tel.: +79637504877; e-mail: victor.zubkov@mail.ru; https://orcid.org/0000-0001-8366-5208

Zheleznyakova Tatyana Viktorovna, Assistant of the Department of Faculty Pediatrics;
Head of the Department of Intensive Care and Neonatal Intensive Care;
tel.: +79054960625; e-mail: mars19501970@list.ru; https://orcid.org/0000-0002-7594-3498

Alhimidi Ali, postgraduate student, pediatrician;
tel.: +7966550864869; e-mail: alialhmidi@outlook.sa; https://orcid.org/0009-0003-0275-7605

Zarytovskaya Natalia Vladimirovna, MD, DMSc, Professor,
Professor of the Department of Propaedeutics of Childhood Diseases;
tel.: +79034468888; e-mail: leda54@mail.ru; https://orcid.org/0009-0009-9178-1634

Zaplatnikov Andrey Leonidovich, MD, DMSc, Professor, Vice-Rector for Academic Affairs, Head of the Department of Neonatology named after Professor V. V. Gavryushov, Professor of the Department of Pediatrics named after Academician G. N. Speransky;
tel.: +79104602873; e-mail: zaplatnikov@mail.ru; https://orcid.org/0000-0003-1303-8318