VITAMIN D LEVEL AND ITS RELATIONSHIP WITH CYTOKINE LEVEL IN BRONCHO-OBSTRUCTIVE SYNDROME IN CHILDREN

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Abstract: The aim of the study was to investigate the effect of vitamin D on the course of broncho-obstructive syndrome in children and to determine its relationship with cytokine status. The study involved 86 patients aged 3 to 15 years with acute obstructive bronchitis, recurrent obstructive bronchitis and bronchial asthma. The patients underwent a single set of diagnostic studies: clinical examination, immunological examination, and a study of vitamin D levels in blood serum. The results of the study showed that vitamin D deficiency was accompanied by the development of secondary immune deficiency.

Keywords: broncho-obstructive syndrome, vitamin D, immune status, children.

BOLALARDA BRONXOOBSTRUKTIV SINDROMDA VITAMIN D DARAJASI VA UNING SITOKIN DARAJASI BILAN O‘ZARO BOG‘LIQLIGI

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Annotatsiya: Tadqiqotning maqsadi bolalardagi bronxoobstruktiv sindromning kechishiga vitamin D ning ta’sirini o‘rganish va uning sitokin statusi bilan o‘zaro bog‘liqligini aniqlashdan iborat edi. Tadqiqotda o‘tkir obstruktiv bronxit, retsidivlanuvchi obstruktiv bronxit va bronxial astma bilan og‘rigan 3 yoshdan 15 yoshgacha bo‘lgan 86 nafar bemor ishtirok etdi. Bemorlarga yagona diagnostik tekshiruvlar majmuasi: klinik tekshiruv, immunologik tekshiruv va qon zardobida vitamin D darajasini aniqlash o‘tkazildi. Tadqiqot natijalari vitamin D yetishmovchiligi ikkilamchi immun yetishmovchiligi rivojlanishi bilan birga kelishini ko‘rsatdi.

Kalit so‘zlar: bronxoobstruktiv sindrom, vitamin D, immun status, bolalar.

УРОВЕНЬ ВИТАМИНА D И ЕГО СВЯЗЬ С УРОВНЕМ ЦИТОКИНОВ ПРИ БРОНХООБСТРУКТИВНОМ СИНДРОМЕ У ДЕТЕЙ

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Аннотация: Цель исследования — изучить влияние витамина D на течение бронхообструктивного синдрома у детей и определить его связь с цитокиновым статусом. В исследовании приняли участие 86 пациентов в возрасте от 3 до 15 лет с острым обструктивным бронхитом, рецидивирующим обструктивным бронхитом и бронхиальной

астмой. Пациентам был проведён единый комплекс диагностических исследований: клинический осмотр, иммунологическое обследование и исследование уровня витамина D в сыворотке крови. Результаты исследования показали, что дефицит витамина D сопровождался развитием вторичного иммунодефицита.

Ключевые слова: бронхообструктивный синдром, витамин D, иммунный статус, дети.

INTRODUCTION

Respiratory pathology, including broncho-obstructive syndrome in young children, remains a pressing issue in modern paediatrics. In the paediatric population, bronchial obstruction occurs in almost 30% of children. Respiratory infections are the most common cause of broncho-obstructive syndrome in young children [1, 2]. It should be noted that the incidence of broncho-obstructive syndrome in children in the first year of life has increased to 50% or more, with 40% of children experiencing at least one episode of bronchial obstruction before reaching school age [2].

The uniformity of clinical symptoms of bronchial obstruction complicates early diagnosis and treatment, which can lead to a protracted and recurrent course. Broncho-obstructive syndrome itself has a characteristic clinical picture, so its diagnosis is straightforward. However, broncho-obstructive syndrome can be a consequence of allergic inflammation, obstructive obstruction, haemodynamic disturbances, and airway remodelling (bronchopulmonary dysplasia, bronchial dystonia, and congenital airway malformations). Broncho-obstructive syndrome most frequently occurs in acute obstructive bronchitis, recurrent obstructive bronchitis, and bronchial asthma. Among the known risk factors for recurrent episodes of broncho-obstructive syndrome — such as a family history of allergies, manifestations of atopy, and eosinophilia — the role of vitamin D and the significance of its deficiency in the predisposition to frequent episodes of bronchial obstruction are currently being actively studied [3–9].

The potential impact of vitamin D on the course of broncho-obstructive syndrome is due to its ability to influence cellular and humoral immunity, thereby reducing inflammation [10–12]. This mechanism is mediated by gene expression and cytokine synthesis. The site of action is vitamin D receptors. The molecular mechanism of action of the highly active metabolite VD — 1,25-dihydroxyvitamin D (1,25(OH)2D), the so-called D-hormone (calcitriol) — is the interaction with specific receptors in tissues — vitamin D receptors — which are widely represented in the body and found in many tissues: T- and B-lymphocytes, macrophages, including pulmonary alveolocytes and bronchial smooth muscle cells [13–16]. It is worth noting that the molecular mechanism underlying vitamin D's non-classical action remains unclear. This is largely due to the insufficient number of randomised trials evaluating the effects of vitamin D on metabolic processes and the course of broncho-obstructive syndrome.

The aim of this study was to investigate the influence of vitamin D levels on the course of broncho-obstructive syndrome in children and to determine its relationship with cytokine status.

MATERIALS AND METHODS

The study involved 86 patients aged 3 to 15 years. Of these, 38 children had acute obstructive bronchitis (Group 1), 15 children had recurrent obstructive bronchitis (Group 2), and 33 children had bronchial asthma (Group 3). Twenty apparently healthy children of the same age served as a control group.

All patients underwent a uniform set of diagnostic tests: clinical examination, immunological blood test, and serum vitamin D level test. The clinical examination included the collection of anamnestic data, including past illnesses, allergy history, the child's premorbid background and an assessment of the general condition at the time of the examination. The immunological study included determination of cytokine levels: IL-4, IL-8, TNF-α, and IFN-γ. These were determined by enzyme-linked immunosorbent assay (ELISA) using reagent kits manufactured by Cytokine LLC (St. Petersburg Research Institute of Highly Pure Biopreparations). Serum vitamin D (25-(OH)D) levels were analysed using an enzyme-linked immunosorbent assay. According to the recommendations of the United States Institute of Medicine, a vitamin D level of ≥20 ng/ml was considered adequate, 11–20 ng/ml was considered insufficient, and ≤10 ng/ml was considered deficiency. The obtained results were processed in the Statistica 10 program; after checking the normality of the distribution using the variation statistics method using Student's t-test, differences were considered statistically significant at $P<0.05$.

RESULTS

Clinical examination revealed that the severity of illness in the examined patients was significantly influenced by an unfavourable premorbid background and comorbidities. In all three observation groups, the most common underlying conditions were anaemia, rickets, and allergic diathesis. In the group of patients with acute obstructive bronchitis, the above-mentioned conditions were more significant. Delayed physical development was observed in 24.6% of patients with bronchial asthma.

Upon admission to the hospital, the main complaints of children with acute obstructive bronchitis and recurrent obstructive bronchitis were cough in 53 (100%), shortness of breath in 53 (100%), increased body temperature in 32 (61.4%) children, decreased appetite in 47 (90.3%), weakness in 40 (76.3%), and sleep disturbance in 41 (91.8%). All children with bronchial asthma complained of cough with mucous sputum — 33 (100.0%), shortness of breath — 33 (100.0%), loss of appetite — 30 (90.8%), lethargy — 31 (95.4%), asthma attacks — 26 (80.0%), sweating — 16 (49.2%), headache — 14 (44.6%). Upon admission to hospital, the leading clinical manifestation of acute obstructive bronchitis was respiratory failure. Weakness was observed in 21 patients (56.0%), and decreased appetite was observed in 30 patients (81.3%). Sleep disturbance, pale skin, and shortness of breath were observed in 38 cases (100.0%). Cyanosis of the nasolabial triangle was present in 12 (32.0%) children. Body temperature was febrile in 7 (18.7%) and subfebrile in 11 (30.6%) patients. Cough was dry in 30 (81.3%) patients and wet in 7 (18.7%) patients. Lung auscultation revealed dry wheezing in 29 (77.3%) patients and wet wheezing in 8 (22.7%) patients, accompanied by harsh breathing. Chest percussion revealed a box-like percussion sound in 30 (88.0%) patients. In children with recurrent obstructive bronchitis, the disease was characterised by an acute (subacute) onset and a cough, initially dry in 90.0% of patients and wet in 10.0%. The cough on admission was paroxysmal, predominantly at night, and transformed into a wet cough by the 5th to 6th day of treatment. Cyanosis of the nasolabial triangle was observed in 25.0% of patients, and dry skin was observed in 70.0% of patients. Auscultatory signs varied and depended primarily on the level of bronchial mucosal damage. Dry rales were detected in 15.0% of children, and moist rales were detected in 85.0% of children.

Based on the results of the cytokine status study, it was found that in children with acute obstructive bronchitis, IL-4 production significantly ($P<0.01$) increased to 18.9±0.3 pg/ml compared to healthy children (Table 1). In patients with recurrent obstructive bronchitis, the IL-4 production indicator was significantly ($P<0.01$) increased to 21.3±0.3 pg/ml, which was 4.6 times

higher than the norm. In patients with bronchial asthma, the IL-4 production rate was increased to 26.7±1.4 pg/ml, which was 5.8 times higher than the norm. When analysing the IL-8 content, its reliable increase was revealed in children of all three groups: with acute obstructive bronchitis up to 32.5±2.8 pg/ml, with recurrent obstructive bronchitis up to 89.5±3.9 pg/ml, with bronchial asthma up to 100.9±7.7 pg/ml ($P<0.01$), which was 5.2 times higher than the norm. Our studies of IFN-γ levels in the examined patients revealed a profound deficiency in their content. Thus, in children with acute obstructive bronchitis, the average serum IFN-γ level was 27.4±1.5 pg/ml ($P<0.05$), with recurrent obstructive bronchitis — 21.4±1.7 pg/ml, and with bronchial asthma — 14.3±1.9 pg/ml, respectively, which is 1.2–1.4–1.6 times lower than the values in practically healthy children (P in all cases from <0.05 to <0.001), and the most significant changes were determined in children with bronchial asthma. Impaired production of IFN-γ, which plays an important role in maintaining homeostasis, is characteristic of the examined patients.

It is known that TNF-α is primarily produced by monocytes and macrophages and performs many critical functions. During inflammation, it activates the endothelium, increases the expression of adhesion molecules on endothelial cells, and promotes leukocyte adhesion to the endothelium. It also activates leukocytes (granulocytes, monocytes, and lymphocytes), and induces the production of other proinflammatory cytokines that act synergistically with TNF-α. In our studies, the level of TNF-α was 3.5 times higher in children with bronchial asthma compared to children in the control group ($P<0.001$), in children with acute obstructive bronchitis it was 48.7±3.5 pg/ml, recurrent obstructive bronchitis — 62.7±3.2 pg/ml versus 28.4±1.5 pg/ml in the control ($P<0.001$), which reflects the increased activity of macrophages involved in maintaining the inflammatory process.

Table 1. Cytokine levels in children in the study groups (M±m)

Indicators	Practically healthy children n=20 (I)	Acute obstructive bronchitis n=38 (II)	Recurrent obstructive bronchitis n=15 (III)	Bronchial asthma n=33 (IV)	P	P_1	P_2
TNF-α (pg/ml)	28.4±1.5	48.7±3.5	62.7±3.2	98.4±4.5	<0.01	<0.001	<0.01
IL-4 (pg/ml)	4.6±0.6	18.9±0.31	21.3±0.34	26.7±1.4	<0.05	<0.001	<0.01
IFN-γ (pg/ml)	34.3±2.7	27.4±1.5	21.4±1.7	14.3±1.9	<0.05	<0.001	<0.01
IL-8 (pg/ml)	19.2±2.4	32.5±2.8	89.55±3.87	100.98±7.67	<0.05	<0.001	<0.01

Note: *P — significance of differences in indicators between groups I and II of patients; P_1 — significance of differences in indicators between groups II and III of patients; P_2 — significance of differences in indicators between groups III and IV of patients.*

Based on the results of the vitamin D3 content in the blood serum, in Group I of patients with acute obstructive bronchitis, 18.5% (12) of patients were found to have vitamin D deficiency, 40.3% (10) with vitamin D insufficiency, and 42% (16) of cases with sufficient vitamin D content. In Group II of patients with recurrent obstructive bronchitis, 26.6% (4) of patients had vitamin D deficiency, 40% (6) had vitamin D insufficiency, and 33.3% (5) of cases had sufficient vitamin D

levels. In Group III of patients with bronchial asthma, 45.4% (15) of patients had vitamin D deficiency, 36.3% (12) had vitamin D insufficiency, and 18.2% (6) of cases had sufficient vitamin D levels (Table 2).

Table 2. Vitamin D levels in study groups

Groups	Vitamin D ≥ 20 ng/ml		Vitamin D 11–20 ng/ml		Vitamin D ≤ 10 ng/ml	
	abs	%	abs	%	abs	%
Group I (acute obstructive bronchitis)	16	42	10	40.3	12	18.5
Group II (recurrent obstructive bronchitis)	5	33.3	6	40	4	26.6
Group III (bronchial asthma)	6	18.2	12	36.3	15	45.4
Control group	10	55	6	30	4	20

The average serum vitamin D (25(OH)-D) levels in the children examined are shown in Figure 1.

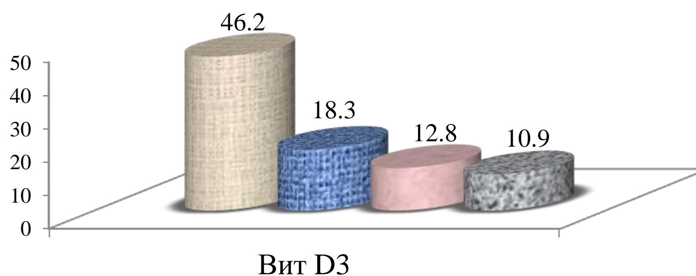

Figure 1. ***Average vitamin D levels in the children examined, ng/ml***

DISCUSSION

As can be seen from Figure 1, the vitamin D3 level in children with bronchial asthma was significantly reduced to 10.9±0.6 ng/ml, which is more than 4.2 times lower than the average values in healthy children (46.2±4.2 ng/ml; P<0.001) and was significantly lower compared to the values in children with acute obstructive bronchitis and recurrent obstructive bronchitis (18.3±3.2 ng/ml and 12.8±1.2 ng/ml, respectively, P<0.01).

Thus, the obtained results indicate a clear association between vitamin D status and the severity of broncho-obstructive syndrome: the most pronounced deficiency was observed in children with bronchial asthma, in parallel with the most significant cytokine imbalance (elevated IL-4, IL-8, TNF-α and reduced IFN-γ). These findings support the role of vitamin D as a modulator of cellular and humoral immunity and suggest its potential value in the complex management of broncho-obstructive conditions in paediatric practice.

CONCLUSION

Based on the data obtained, the following conclusions can be drawn:

1. The severity of broncho-obstructive syndrome is associated with a complicated premorbid background, comorbidities, and previous illnesses, which significantly worsen the underlying pathology. In broncho-obstructive syndrome, serum cytokine levels vary depending on the underlying disease and are characterised by significant increases in IL-4, IL-8, and TNF-α, and a decrease in IFN-γ.
2. Vitamin D deficiency is accompanied by the development of secondary immune deficiency, manifested by decreased phagocytic activity and reduced interferon production. The greatest

changes in immune status are observed in patients with bronchial asthma, characterised by increased IL-4, IL-8, and TNF-α and decreased IFN-γ.

3. The increase in the severity of broncho-obstructive syndrome is directly proportional to the decrease in the level of vitamin D metabolites in the blood serum, which is clearly evident in children with bronchial asthma.

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