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Clinical and Immunological Study of The Cytokine Profile of Auxiliary T-Lymphocytes in Children Up To 3 Years of Age with Congenital Cleft Lip and Palate in Community Aquired Pneumonia

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Abstract: OBJECTIVE: To evaluate the synthesis of major cytokines characteristic of Th1/Th2/Th17 immune responses in children with congenital cleft lip and palate in acute CAPeumonia.

MATERIAL AND METHODS: The present study included 46 children with an established diagnosis of community acquired pneumonia (22 children with CCLP and 24 children without CCLP formed the comparison group) and 20 practically healthy children formed the control group. The concentration of interleukins and interferon-γ in the blood serum was determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA) using the test systems of Vector-Best JSC (Novosibirsk, Russia).

RESULTS: It was found that in groups of children with CAP, both with congenital cleft lip and without congenital pathology, there was a significant increase in the content of IL-17A, IL-4 in the peripheral blood and a deficiency of IFN-γ in young children, which indicates an imbalance in the immune response that can affect the course and severity of the infection.

Keywords: Early childhood, cleft lip and palate, pneumonia, interferon, interleukin, serum, imbalance.

Introduction: Congenital cleft lip and palate (CLLP) is a severe malformation of the face and jaws, which leads to anatomical and functional disorders. The problem of changes in the immune system in children with CLLP has been little studied, although it is known that secondary immunodeficiencies may occur in children with congenital malformations (CM) [1].

Cleft lip, with or without cleft palate, is one of the most common congenital malformations, occurring in an average of 1 in 1000 newborns. Cleft lip and/or palate is incredibly variable in terms of phenotype, and treatment options for patients are constantly evolving [3].

According to various authors, “frequently and long-term ill” people make up from 20 to 65% of the child population from the dispensary group, with a high frequency of recurrent respiratory infections against the background of various immune system disorders [2,4].

Recurrent episodes of acute respiratory infections (ARI), typical for children with congenital cleft lip and palate (CLPP), indicate possible disorders in the functioning of their immune system. Solving the problem of high frequency of respiratory diseases in this group of patients, as well as the choice of optimal therapeutic tactics, requires a comprehensive pathogenetic approach, which provides for a detailed assessment of the state of the immune system. Based on the obtained results, it is advisable to develop new immunotherapeutic strategies aimed at eliminating the identified immune dysfunctions [5] .

At the same time, the nature of immune disorders at individual stages of the inflammatory process has not been studied fully enough and is interpreted ambiguously. Therefore, it is of interest to study the cellular and cytokine links of immunity, since they regulate the strength, duration of the immune response and the nature of the inflammatory process, ensuring positive and negative immunoregulation.

As stated above, the formation and development of the immune system is a process that is determined by the interaction of the body with environmental factors – antigens.

METHODS

Based on the above, in this study, the features of the synthesis of interferon-gamma (IFNγ), proinflammatory interleukin 17A (IL-17A) and anti-inflammatory interleukin - 4 (IL -4) were studied in 46 children before and after treatment. Of these, those with an established diagnosis of acute pneumonia (22 children with CCLP and 24 children without CCLP formed the comparison group) and 20 practically healthy children formed the control group.

Immunological studies of patients included in the survey were performed in the laboratory of immunoregulation of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan.

The concentration of interleukins and interferon-γ in the blood serum was determined by the enzyme-linked immunosorbent assay (ELISA) method using the test systems of Vector-Best JSC (Novosibirsk, Russia). The quantitative assessment of the results was carried out using a calibration curve constructed on a series of standards with concentrations from 7.8 to 500 pg/ml. Optical density was recorded at 450 nm on a BioTek ELx800 microplate reader. A four-parameter logistic model (4PL) was used to approximate the calibration dependence; values outside the linear part were interpolated taking into account the inverse transformation. Intraday precision was assessed on control samples (low/medium/high concentration), obtaining intraday CV ≤ 10%, interday CV ≤ 15%. Curves were plotted and concentrations were interpolated in GraphPad Prism.

Statistical processing of the obtained data was carried out using the computer program " Statistica 6.0".

The data were statistically processed using conventional approaches, the results are presented as sample mean (M) and standard error of the mean (m); median (Me), characterizing the central tendency, and upper and lower quartiles characterizing the spread of indicator values in 50% of respondents (Q1-Q3), where Q1 is the 25% percentile, Me is the 50% percentile, Q3 is the 75% percentile. The reliability of differences in the mean values (P) of the compared indicators was assessed using the Student's criterion (t).

RESULTS AND DISCUSSION

The age of the examined children corresponded to the third critical period of the immune system development. At this time, the child's contacts with the outside world (freedom of movement, socialization) significantly expand. The primary immune response (IgM synthesis) to many antigens is preserved. At the same time, the immune reactions begin to switch to the formation of IgG antibodies. The local immune system remains immature. Therefore, children remain sensitive to viral and microbial infections. Children are prone to recurrent viral and microbial inflammatory diseases of the respiratory system, ENT organs. According to immunobiological characteristics, a significant proportion of children in their second year of life are not ready for the conditions of staying in a children's group [6].

The study of the level of cytokines regulating individual development, physiological functions and protective reactions of the body [8] allows obtaining information on the functional activity of cells, the stage of the inflammatory process and its severity, the ratio of the processes of activation of cytokine-producing T-lymphocytes, which is of great diagnostic and prognostic value [7]. In this regard, the interest of the present study is the study of serum levels of IFN -γ, IL -4, as well as regulatory IL -17 A in children with and without CLP in renal failure at the age of 1 to 3 years.

The results of immunological studies in this cohort of children before the start of treatment are presented below, in Table 1.

Table 1.
Serum cytokine levels in groups of children aged 1 to 3 years before treatment

Indicator	M±m , pg/ml	Me [Q1; Q3]	Min , pg/ml	Max , pg/ml
Control group, n =20				
IFNγ	13.42±0.76	13.45 [9.80 ; 16.01]	9.32	19.35

IL-17A	7.59±0.38	7.20 [6.28 ; 8.9 2]	5.30	10.52
IL – 4	5.18±0.30	4.91 [4.05 ; 5.98]	3.14	8.30
1st main group (children with VRGN+CAP), n = 22				
IFNγ	7.71±0.33***	7.70 [6.57 ; 9.12]	4.93	9.91
IL-17A	46.19±1.65***	48.25 [38.77 ; 51.37]	31.50	58.12
IL – 4	30.48±1.18***	30.50 [27.87 ; 33.57]	20.44	42.30
2nd comparison group (children with CAP), n = 24				
IFNγ	8.73±0.38***	8.50 [7.52 ; 10.52]	5.22	11.70
IL-17A	30.62±1.21***	31.15 [26.07 ; 34.15]	21.64	41.71
IL – 4	18.24±1.24***	17.65 [13.35 ; 23.91]	9.45	24,23

*Note: * - reliable compared with the control group data (* - P < 0.05, ** - P < 0.01, *** - P < 0.001). Me – median, Q 1 (percentile) – 25%, Q 3 (percentile) – 75%.*

IFNγ occupies a special place in the interferon family. IFNγ can also be synthesized by myeloid cells, including macrophages and dendritic cells. Activation of T cells via TCR requires significantly more time, as a result of which the maximum production of IFNγ due to it is achieved 48-72 hours after cell stimulation [9].

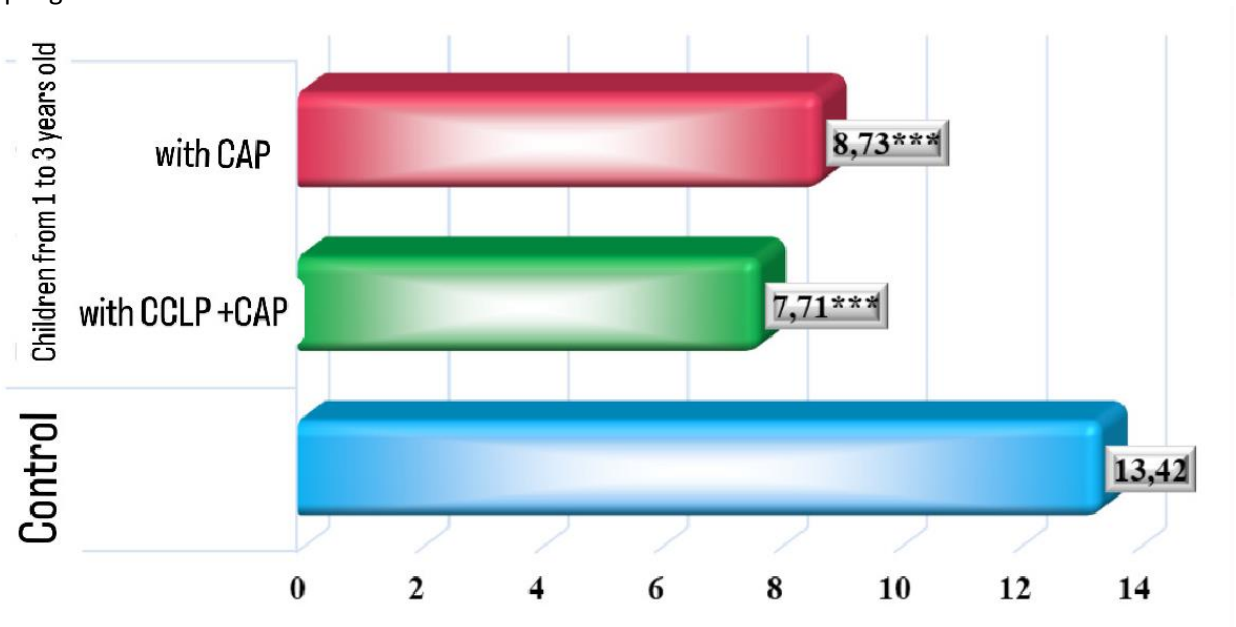

Figure 1. Serum IFN-γ levels in examined children aged 1 to 3 years before treatment.
*Note: * - significant compared to control group data (* - P < 0.05, ** - P < 0.01, *** - P < 0.001).*

The analysis of serum IFN-γ content revealed significant values in all examined children with CAP aged 1 to 3 years. It was revealed that in the group of children with congenital cleft lip and CAP, IFN-γ synthesis was significantly reduced by 43% and averaged 7.71±0.33 pg/ml, with an individual range of 4.93 to 9.91 pg/ml (P<0.001), whereas in the group of children without congenital pathology with CAP, the level of this indicator averaged 8.73±0.38 pg/ml, with an individual range of 21.64 to 41.71 pg/ml (P<0.001) against the control values of children of similar age, which averaged 13.42±0.76 pg/ml (Fig. 1).

Our results of reduced IFN-γ levels in groups of children aged 1 to 3 years with CAP, as well as in groups of infants, indicate various aspects of the immune

response and the state of the body. Thus, in the group of children with CLP with CAP and without this facial anomaly, reduced IFN-γ levels may indicate weak antiviral protection, and its deficiency makes the body more vulnerable to viral infections, which is associated with immune system disorders.

IL -17A, being a signature effector cytokine of Th17, primarily induces the activation and recruitment of neutrophils to sites of infection and inflammation [10]. In the airway epithelium, it mediates neutrophil chemotaxis through the induction of chemokines CXCL1 and CXCL5.

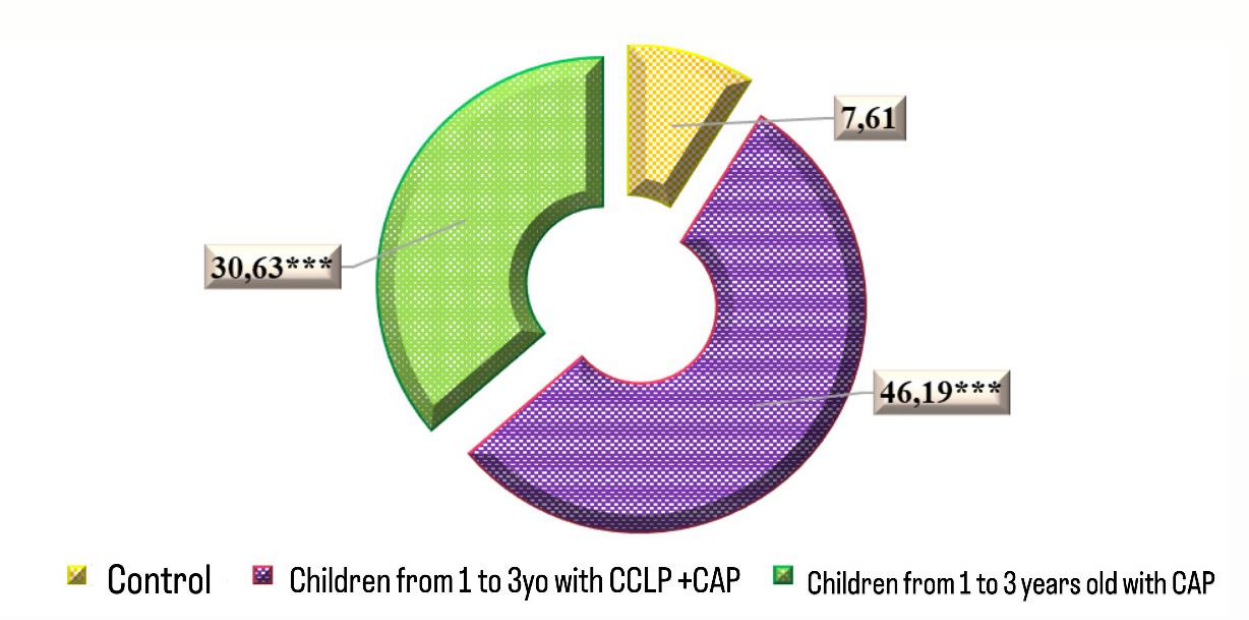

Figure 2. Serum IL-17A level in examined children aged 1 to 3 years before treatment.

*Note: * - significant compared to control group data (* - P < 0.05, ** - P < 0.01, *** - P < 0.001).*

The study of the serum concentration of IL-17A, shown in Fig. 2, revealed a significant increase in all groups of children with CAP. Thus, the maximum level of IL-17A was detected in the group of children with congenital cleft lip and cleft palate with CAP, which exceeded the normative values by more than 6 times, and on average amounted to 46.19±1.65 pg/ml (P<0.001), with an individual range from 31.50 to 58.12 pg/ml, while in the group of children with CAP without congenital pathology this indicator exceeded the normative values by 4 times with an average value of 30.62±1.21 pg/ml (P<0.001), with a range from 21.64 to 41.71 pg/ml versus the control of 7.59±0.38 pg/ml.

The established increase in the level of IL-17A in CAP in children aged 1 to 3 years is explained by the fact that the immune system is in the stage of active formation and adjustment. This age is characterized by a high frequency of respiratory infections, and the immune response can be more active and inflammatory, IL-17A can be activated in response to bacterial or fungal infections, which are often the causes of pneumonia. Th17 cells producing IL-17A play a role in the fight against these pathogens, which is typical for the immune response to infection. And children with CLP are a special risk group, due to the vulnerability of the mucous membranes of the oral and respiratory systems of the body from birth.

As noted above, the main producers of IL-4 are Th2 cells, but in addition to the main producer cells, mast cells, basophils, eosinophils, NK and NKT cells, as well as dendritic cells (DC-2) also produce it, mainly spontaneously.

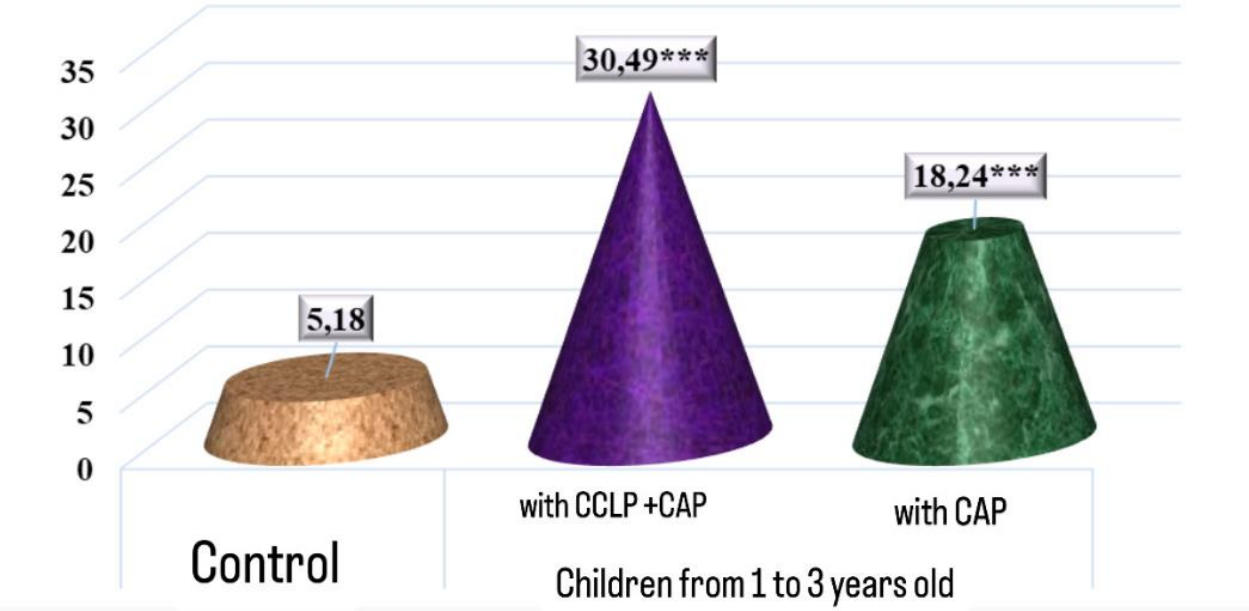

Figure 3. Serum IL-4 level in examined children aged 1 to 3 years before treatment.
*Note: * - significant compared to control group data (* - P < 0.05, ** - P < 0.01, *** - P < 0.001).*

Analysis of serum IL-4 concentration in children aged 1 to 3 years with CAP in all groups revealed a reliable increase. It was found that in the group of children with congenital cleft palate the level of the studied anti-inflammatory cytokine was higher than the standard values by more than 5.9 times, which on average amounted to 30.48±1.18 pg/ml (P<0.001), with an individual range from 20.44 to 42.30 pg/ml, in the group of children without congenital facial pathology with CAP this indicator was increased by 3.5 times, with an average value of 18.24±1.24 pg/ml (P<0.001), with an individual range from 9.45 to 24.23 pg/ml against the control values in healthy children 5.18±0.30 pg/ml (Fig. 3).

The obtained results allow us to assume that it is possible that the increased values of IL-4 in children with CCLP in CAP are associated with features in the immune system that can affect the regulation of cytokines, including IL-4. Increased levels of IL-4 are associated with activation of the immune system in response to infections. However, an increase was also found in the group of children with CAP without congenital anomalies, which means that increased synthesis of this anti-inflammatory cytokine is associated with stimulation and activation of producer cells to the site of inflammation in the respiratory system.

Thus, the immunological studies conducted among children aged 1 to 3 years, to study the cytokine profile in a comparative aspect in immunocompromised children with congenital cleft lip and in children without congenital facial defects with CAP, in comparison with the state of the immune system in conditionally healthy children of the same age, revealed hypersecretion of key mediators of the Th2 and Th17 immune response (IL-4 and IL-17A) and a deficiency in the synthesis of the key antiviral cytokine of the Th1 immune response (IFNγ) in all groups of children with CAP. It has been established that a more pronounced imbalance in the group of children with congenital cleft lip, which is probably explained by the presence of an anatomical defect of the upper lip and palate (open gates for the penetration of infections) and a combined immunodeficiency of a secondary nature aggravate infectious and inflammatory processes in the mucous membranes of the oral cavity and upper respiratory tract, which manifest as recurrent exacerbations of chronic infectious and inflammatory diseases of the oral cavity, respiratory tract and ENT organs even outside the period of acute clinical manifestations of diseases.

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