FEATURES OF CYTOKINE REGULATION IN CHILDREN WITH ACYANOTIC CONGENITAL HEART DEFECTS

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АБСТРАКТ

Врожденные пороки сердца (ВПС) без цианоза — это группа аномалий сердца, которые не приводят к смешению крови между системным и легочным кругами с такой степенью, чтобы вызвать синюшность или цианоз.

С целю изучение концентрации цитокинов у детей с врожденными пороками сердца бледного типа было обследовано 28 детей с установленным диагнозом ВПС белого типа. Группу контроля составили 28 практически здоровых детей, аналогичного возраста. Концентрацию интерлейкинов и фактора некроза опухолей альфа (IL-6, IL-8, TNF-α) в сыворотке крови определяли методом твердофазного иммуноферментного анализа с использованием тест-систем АО «Вектор-Бест» (Новосибирск, Россия).

Установлена гиперсекреция IL-6, IL-8, TNF-α у пациентов с ВПС. Увеличение содержания изученных медиаторов иммунного ответа может быть защитной реакцией организма на стресс и повреждение тканей, однако длительная экспрессия может привести к патологическим изменениям и влиять на развитие и прогрессирование ВПС.

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КЛЮЧЕВЫЕ СЛОВА: врожденные пороки сердца, дети, без цианоз, цитокины, сыворотка, дисбаланс.

ABSTRACT

Acyanotic congenital heart defects (CHDs) are a group of cardiac anomalies that do not cause mixing of systemic and pulmonary blood to a degree sufficient to produce cyanosis.

To investigate cytokine concentrations in children with acyanotic congenital heart defects, 28 children with a confirmed diagnosis of acyanotic CHD were examined. The control group consisted of 28 practically healthy children of comparable age. The concentrations of interleukins and tumor necrosis factor alpha (IL-6, IL-8, TNF-α) in blood serum were measured using solid-phase enzyme-linked immunosorbent assay (ELISA) with test systems manufactured by Vector-Best (Novosibirsk, Russia).

Hypersecretion of IL-6, IL-8, and TNF-α was detected in patients with CHD. The increased levels of the studied immune response mediators may represent a protective reaction of the body to stress and tissue damage; however, prolonged expression may lead to pathological alterations and influence the development and progression of CHDs.

The elevated concentrations of the investigated immune mediators may serve as a protective mechanism, yet their sustained expression can contribute to pathological changes and affect the course and progression of congenital heart defects.

KEYWORDS: congenital heart defects, children, acyanotic, cytokines, serum, imbalance

RELEVANCE:

Congenital heart defects (CHD) without cyanosis are a group of cardiac anomalies that do not cause blood mixing between the systemic and pulmonary circulations to an extent that would result in cyanosis. These defects can take various forms and vary in severity.

The study of interactions between the immune system and pathological processes has limited applicability in the context of congenital heart defects, since the primary causes of these defects are related to the development of the

cardiovascular system during the embryonic period rather than to immune mechanisms.

However, immunological mechanisms may play some role in the formation of CHD. For example, some studies suggest a possible link between CHD and immune factors, such as maternal immune responses to infections during pregnancy or autoimmune processes. Nevertheless, these associations remain under active investigation, and the precise mechanisms by which immune factors may influence the development of acyanotic CHD are not yet fully understood.

It is known that most diseases are associated with elevated levels of pro-inflammatory cytokines. It is believed that chronic inflammatory processes may be sustained by an imbalance between pro- and anti-inflammatory cytokines. Cytokines are biologically active factors produced by numerous cells from various tissues and organs; they are secreted by cells during their life processes in response to external stimuli [24].

The main cells synthesizing and secreting immunoregulatory cytokines in the body are helper T-lymphocytes (Th), which differ in phenotype and the spectrum of marker cytokines they produce: Th0 (IL-2), Th1 (IL-2, IFN-γ, TNF-α, and TNF-β) (cellular immune response), and Th2 (IL-4, IL-5, IL-6, IL-9, IL-13) (humoral immune response) [6]. Since cytokines and their receptors are synthesized in response to antigen stimulation of cells, the overall activity level of the cytokine system determines the nature of the body's response to physiological and pathogenic stimuli [21].

According to current understanding, an imbalance between Th1 and Th2 cytokines may play a role in the immunopathogenesis of virtually all diseases, both infectious and non-infectious [20].

Pro-inflammatory cytokines (IL-1, IL-6, IL-12, TNF-α, interferons, chemokines, IL-8, etc.) stimulate the inflammatory response and help eliminate damaged cells and viruses. Elevated levels of these cytokines in the blood reflect the activity and severity of the inflammatory process.

Inflammation plays an important role in cardiovascular diseases, and inflammatory markers can predict future cardiovascular events [11].

Currently, according to the literature, there is a growing body of evidence indicating that the levels of anti-inflammatory cytokines in the blood show a significant positive association with various types of CHD [14].

OBJECTIVE: To study cytokine concentrations in children with acyanotic congenital heart defects.

MATERIALS AND METHODS

This study included 28 children diagnosed with acyanotic CHD. The control group consisted of 28 practically healthy boys and girls of similar age. The participants were aged from 1 to 12 years.

Immunological studies in the examined children were conducted at the Laboratory of Immunoregulation, Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan.

The concentrations of interleukins and tumor necrosis factor-alpha (IL-6, IL-8, TNF-α) in peripheral blood serum were determined using a solid-phase enzyme-linked immunosorbent assay (ELISA) with test systems from JSC “VECTOR-BEST” (Novosibirsk, Russia). Quantitative assessment of the results was performed by constructing a calibration curve, reflecting the relationship between optical density and concentration for the standard antigen, which allowed comparison with the studied samples.

Statistical analysis of the obtained data was performed using the computer program “Statistica 6.0.”

The data were statistically processed using standard approaches. Results are presented as the sample mean (M) and standard error of the mean (m); the median (Me), representing the central tendency; and the upper and lower quartiles, reflecting the spread of values in 50% of respondents (Q1–Q3), where Q1 is the 25th percentile, Me is the 50th percentile, and Q3 is the 75th percentile. The significance of differences between mean values (P) of the compared parameters was assessed using Student’s t-test.

RESULTS AND DISCUSSION

Pro-inflammatory mechanisms of heart failure are mediated by various cytokines, which can induce cardiomyocyte hypertrophy, apoptosis, fibrosis, and ultimately lead to adverse cardiac remodeling. In accordance with the study objective, the first stage of our immunological investigations involved determining the serum levels of a range of pro-inflammatory cytokines in the group of children with acyanotic CHD. The obtained results are presented in Table 4.1 below.

Table 4.1

Serum Levels of Pro-Inflammatory Cytokines in Examined Children with Acyanotic CHD

Indicator	**M±m, pg/mL**	**Me [Q1; Q3]**	**Min, pg/mL**	**Max, pg/mL**
Control group, n=28				
IL-6	6,25±0,43	5,90 [4,12; 8,82]	2,51	9,70
IL-8	12,66±0,60	12,45 [9,70; 15,67]	9,11	19,13
TNF-α	15,17±0,49	15,22 [13,3; 17,28]	10,63	19,74
Congenital heart defects of the acyanotic type (without cyanosis), n=28				
IL-6	22,08±1,09***	21,90 [17,3; 25,2]	12,30	33,12
IL-8	46,82±1,86***	47,30 [37,47; 53,72]	29,71	63,52
TNF-α	56,53±2,82***	53,25 [45,87; 66,80]	32,93	86,52

Note: * — significant compared to the control group (* P<0.05, P<0.01, * P<0.001). Me — median; Q1 — 25th percentile; Q3 — 75th percentile.

Members of the IL-6 family modulate the immune response and inflammatory activity, and subsequently participate in the development of cardiovascular diseases [5,7,9].

Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine that can be produced by activated T cells, B cells, monocytes, and malignant cells. Macrophages, adipocytes, hematopoietic cells, and endothelial cells are also cellular sources of IL-6 [16].

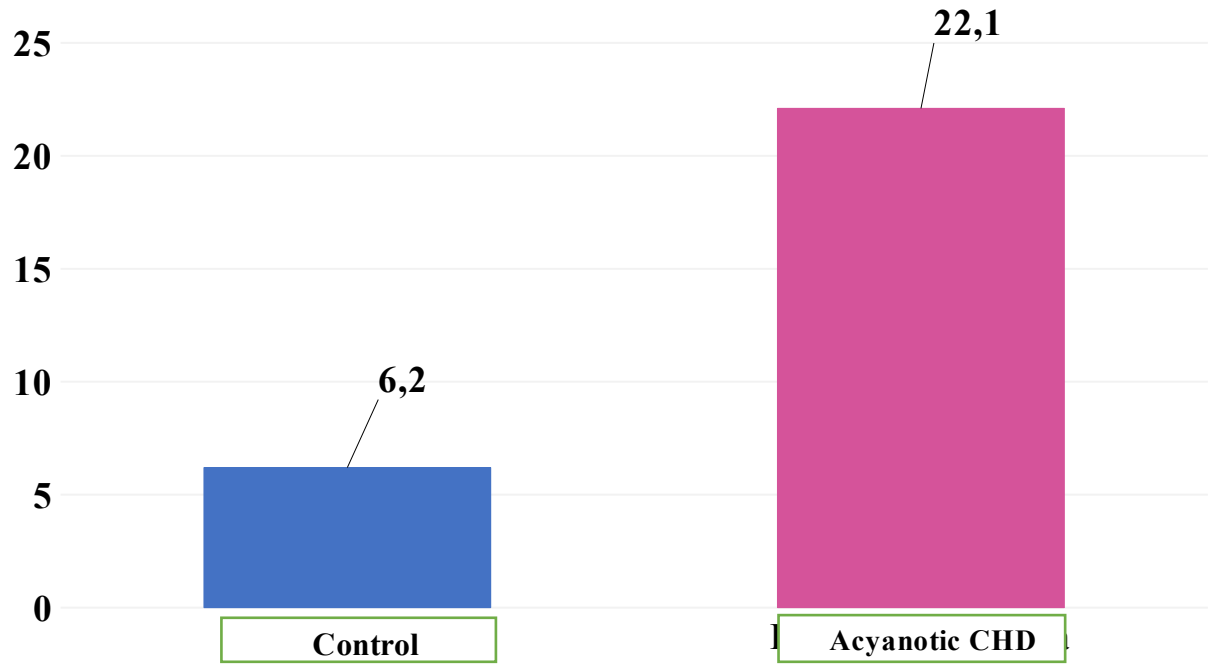

Figure 4.1. IL-6 Levels in Children with Acyanotic CHD and in the Control Group.

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

Analysis of the obtained results for IL-6 in the serum of children with acyanotic CHD revealed statistically significant differences compared to healthy children in the control group. The serum IL-6 level in children with acyanotic CHD was increased 3.5-fold, with a mean value of 22.1 ± 1.09 pg/mL, whereas in the control group it averaged 6.2 ± 0.44 pg/mL (P<0.001).

IL-6 is a cytokine that plays an important role in the immune system and inflammatory processes. Elevated IL-6 levels may be associated with various

pathological conditions, including congenital heart defects (CHD). The obtained results suggest possible links between IL-6 and CHD:

1. Inflammatory response: CHD may trigger inflammatory reactions in cardiac tissue and blood vessels. IL-6 is a key mediator of inflammation and can be produced by immune cells in response to tissue damage.

2. Cardiac remodeling: CHD can lead to abnormal remodeling of cardiac tissue, including myocardial hypertrophy. IL-6 may stimulate remodeling processes in the heart and contribute to the development of hypertrophy.

3. Immune regulation: IL-6 also plays a critical role in immune regulation and can affect various aspects of the immune response, including activation and function of immune cells. Dysregulation of immune responses may be associated with CHD development, and elevated IL-6 levels may indicate an imbalance in the immune system.As is known, chemokines are a family of cytokines that induce directed migration of leukocytes along a concentration gradient, leading to the accumulation of migrating cells at the chemokine source [19]. Therefore, the next stage of our research was to study serum IL-8 levels, thereby identifying the role of this immune response mediator in CHD.

IL-8 is a pro-inflammatory cytokine, or chemokine (CXCL8), produced by various cell types, including endothelial cells, peripheral blood monocytes, and vascular smooth muscle cells. IL-8 is encoded by IL8 or CXCL8 [15]. Studies by Kim D.-H. et al. (2013) and Takami M. et al. (2002) in patients and experimental cell culture models [6,12] have shown that IL-8 is involved in the pathogenesis of cardiovascular diseases, including coronary artery disease (CAD) [12], myocardial infarction (MI) [13], strokes [17], and other conditions. In cardiovascular diseases, IL-8 participates in all stages of atherosclerosis and the development of CAD [1,4]. According to the described mechanisms, under normal physiological conditions, macrophages, endothelial cells, and epithelial cells produce IL-8 in response to infection or tissue damage. One of the functions of IL-8 is to induce chemotaxis of granulocytes, primarily neutrophils, to the affected area [2]. IL-8 signaling in

vascular endothelial cells stimulates cell proliferation, survival, and migration [8], ultimately leading to the formation of new blood vessels [10].

To determine the role and involvement of the above-mentioned chemokine under normal conditions and in various types of CHD, we studied serum IL-8 levels as one of the key mediators of the immune response and an activator of the angiogenic response. The comparative results obtained during the study are presented in Table 4.1 and Figure 4.2.

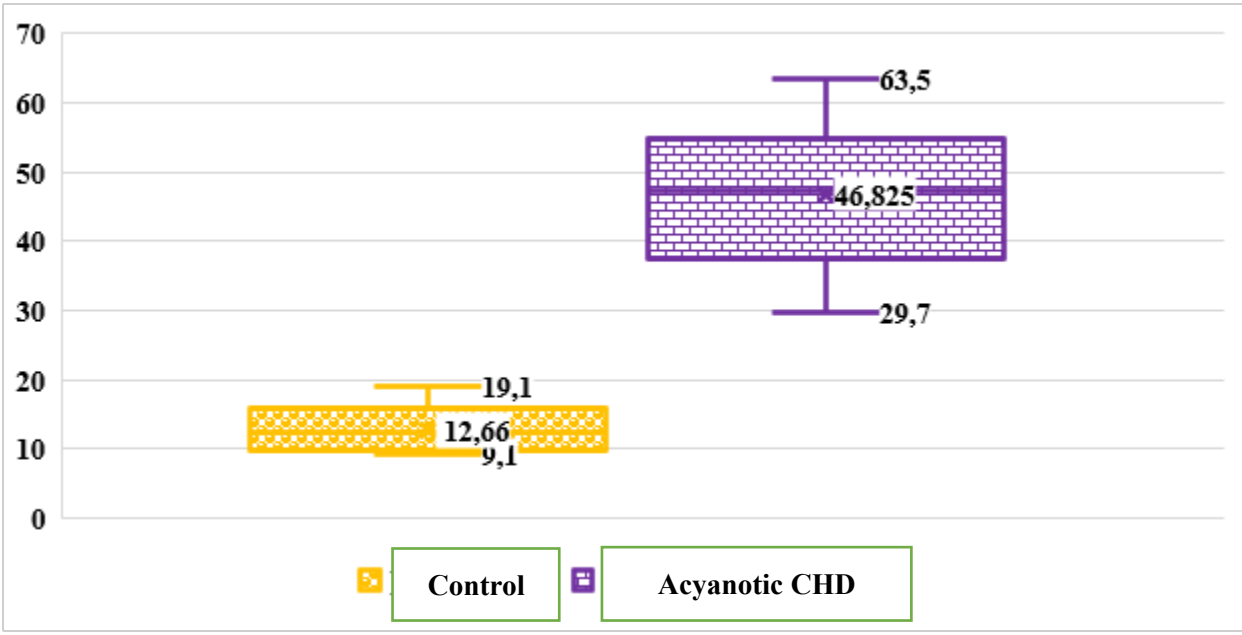

Figure 4.2. Serum IL-8 Levels in the Groups of Examined Children.

Analysis of the results revealed relatively high IL-8 levels in the group of children with acyanotic CHD. The serum concentration of this chemokine in the main group was increased 3.7-fold, with a mean value of 46.82 ± 1.86 pg/mL and an individual range of 29.7 to 63.5 pg/mL, whereas in the group of practically healthy children, the mean level was 12.66 ± 0.60 pg/mL ($P<0.001$). We hypothesize that the elevated levels of interleukin-8 (IL-8) in congenital heart defects may be explained by several factors:

1. Inflammation and immune activation: CHD triggers inflammatory responses in cardiac tissue and blood vessels. IL-8 is a cytokine that plays a key role in attracting and activating inflammatory cells such as neutrophils and macrophages. Elevated IL-8 levels may reflect activation of the inflammatory

process resulting from the congenital heart defect. 2. Angiogenesis: IL-8 is also involved in the regulation of angiogenesis—the formation of new blood vessels. Increased IL-8 levels may indicate enhanced activation of angiogenic mechanisms aimed at compensating for vascular defects.

3. Regulation of the immune response: IL-8 can influence the immune response by regulating the activity of immune cells and the production of pro-inflammatory cytokines. Elevated IL-8 levels may indicate an imbalance in the immune system, which is likely associated with acyanotic CHD.

One of the key pro-inflammatory cytokines widely studied as a potential biomarker associated with heart failure is TNF-α [3].

Tumor necrosis factor alpha (TNF-α) is a member of the family of immunologically significant proteins and a pro-inflammatory cytokine with a broad range of activity. The main producers of TNF-α are monocytes and macrophages. It is also secreted by neutrophils, endothelial and epithelial cells, eosinophils, mast cells, and B- and T-lymphocytes when involved in the inflammatory process [23].

Under normal conditions, TNF-α plays a fundamental physiological role in immune regulation. However, in certain cases, it can exert pathological effects, contributing to the development and progression of inflammation, microvascular hypercoagulation, hemodynamic disturbances, and metabolic wasting (cachexia) in various human diseases of both infectious and non-infectious origin [18].

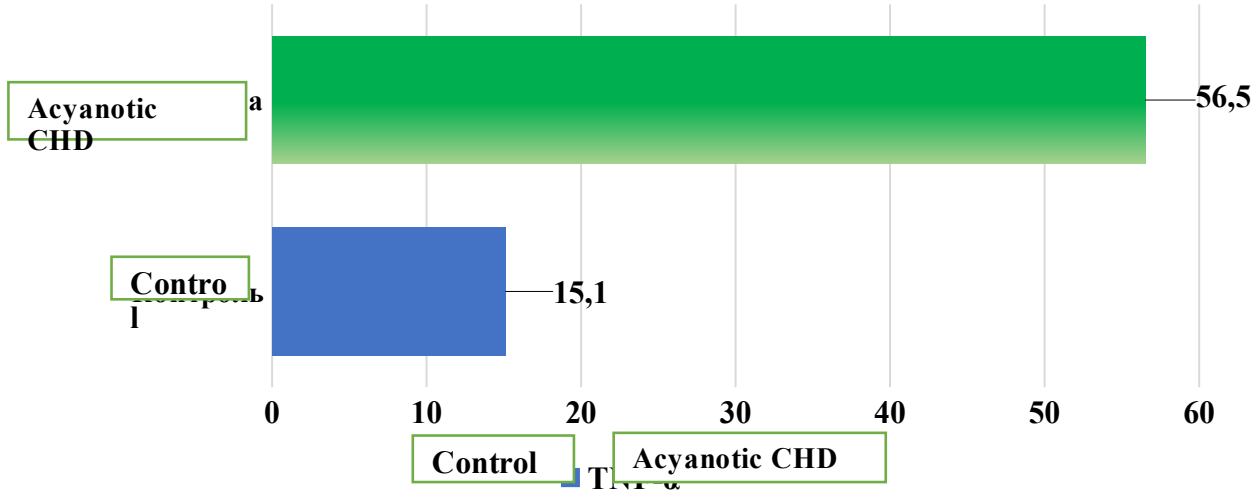

Figure 4.3. TNF-α Levels in Children with Acyanotic CHD and the Control Group. Note: * — significant compared to the control group (* P<0.05, P<0.01, * P<0.001).

Comparative assessment of serum TNF-α levels, shown in Figure 4.3, revealed statistically significant results in the group of children with acyanotic CHD. TNF-α synthesis in children with acyanotic CHD increased 3.7-fold, with a mean value of 56.53 ± 2.82 pg/mL and a range of 32.9 to 86.5 pg/mL, whereas in healthy children, the mean level was 15.17 ± 0.49 pg/mL (P<0.001) (Table 4.1).

Elevated serum TNF-α levels in the group of children with acyanotic CHD likely indicate the development of cardiac cachexia in children with chronic congestive heart failure or chronic hypoxemia, and may reflect several factors:

1. Inflammation: TNF-α is an important mediator of inflammation and can be produced by immune cells in response to tissue damage.

2. Cardiac remodeling: TNF-α may be involved in remodeling processes and contribute to the development of fibrosis and hypertrophy. Elevated TNF-α levels may reflect its participation in pathological changes in cardiac tissue in congenital heart defects.

3. Immune regulation: TNF-α is one of the key cytokines regulating the immune response, and its elevated levels may indicate an imbalance in this system.

CONCLUSION

Thus, the elevated levels of the studied pro-inflammatory cytokines in acyanotic CHD result from a complex interplay between pathological changes in the heart, stress conditions, immune system activation, and compensatory mechanisms of the body. Therefore, further research is required to fully understand the mechanisms of this process and the potential use of pro-inflammatory cytokines in the diagnosis and treatment of CHD.

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