THE SIGNIFICANCE OF PRO-INFLAMMATORY IMMUNE MEDIATORS IN THE PATHOGENESIS OF VARIOUS FORMS OF CONGENITAL HEART DEFECTS IN CHILDREN

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ABSTRACT

Congenital heart defects (CHDs) account for about 30% of all developmental anomalies in children and are among the most common defects today. The study included 52 children with a confirmed diagnosis of congenital heart defects (CHDs), classified as acyanotic (28 patients) and cyanotic (24 patients). The control group consisted of 28 practically healthy children of comparable age. The serum concentrations of interleukins and tumor necrosis factor alpha (IL-6, IL-8, TNF-α) were measured using solid-phase enzyme-linked immunosorbent assay. Hypersecretion of IL-6, IL-8, and TNF-α was observed in patients of both CHD groups. 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 changes and affect the development and progression of CHDs.

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 can lead to pathological changes and influence the development and progression of CHDs. Further research and clinical observations are necessary for a more precise understanding of the role of these mediators in CHD pathology.

KEYWORDS: congenital heart defects, children, cyanosis, cytokines, serum, imbalance.

АБСТРАКТ

Врожденные пороки сердца (ВПС) составляют около 30 % от всех аномалий развития у детей и являются одними из самых распространенных пороков в настоящее время. Для обследование включены 52 ребенка с установленным диагнозом ВПС белого (28 пациентов) и синего (24 пациентов). 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.

Установлена гиперсекреция IL-6, IL-8, TNF-α у пациентов обеих групп с ВПС.Увеличение содержания изученных медиаторов иммунного ответа может быть защитной реакцией организма на стресс и повреждение тканей, однако длительнаяэкспрессия может привести к патологическим изменениям и влиять на развитие и прогрессирование 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. Для более точного понимания роли этих медиаторов в патологии при ВПС необходимы дальнейшие исследования и клинические наблюдения.

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

RELEVANCE: Congenital heart defects (CHDs) account for about 30% of all developmental anomalies in children and are among the most common defects today [1,2,5,11]. According to statistics from various countries around the world, between 0.6% and 1.4% of infants are born with CHDs [9]. As their prevalence increases, there is a tendency toward a higher proportion of more severe, combined CHDs with unfavorable outcomes already in the first months of life [3,4,8].

The most common defects are as follows: ventricular septal defect (VSD) – 28.3%; atrial septal defect (ASD) – 10.3%; pulmonary artery stenosis – 9.8%; Tetralogy of Fallot (TOF) – 9.7%; aortic stenosis – 7.1%; aortic coarctation – 5.1%; transposition of the great arteries – 4.9%; other defects include tricuspid valve hypoplasia, patent ductus arteriosus (PDA), and total anomalous pulmonary venous return [9]. More than 90 variants of CHDs and numerous combinations have been observed[6].

The study of the interaction between the immune system and pathological processes has limited application in the context of congenital heart defects (CHDs), since the primary causes of these defects are related to the development of the cardiovascular system during the embryonic period, rather than immune mechanisms.

However, immunological mechanisms may play some role in the formation of CHDs. For example, some studies suggest a possible association between CHDs and immune factors, such as maternal immune responses to infections during pregnancy or autoimmune processes. These associations are still under active investigation, and the exact mechanism by which immune factors may influence CHD development is not yet fully understood.

Based on the above, the objective of the present study was to comparatively determine the role of serum levels of key pro-inflammatory immune response mediators (IL-6, IL-8, TNFα) in children with acyanotic and cyanotic CHDs.

OBJECTIVE: To study the serum concentration of pro-inflammatory immune response mediators (IL-6, IL-8, TNFα) in congenital heart defects (CHDs).

MATERIALS AND METHODS: This study included 52 children with a confirmed diagnosis of CHDs: acyanotic (28 patients) and cyanotic (24 patients). The control group consisted of 28 practically healthy boys and girls of comparable age. The participants were aged between 1 and 12 years.

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

The serum concentrations of interleukins and tumor necrosis factor alpha (IL-6, IL-8, TNF-α) in peripheral blood were measured using a solid-phase enzyme-linked immunosorbent assay (ELISA) with test systems from Vector-Best JSC (Novosibirsk, Russia). Quantitative evaluation of the results was performed using a calibration curve, reflecting the relationship between optical density and the concentration of the standard antigen, which allowed comparison with the tested samples.

Statistical analysis of the obtained data was carried out using the Statistica 6.0 software. The data were statistically processed using standard approaches, and results are presented as the 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 values among 50% of respondents (Q1–Q3), where Q1 is the 25th percentile, Me – 50th percentile, and Q3 – 75th percentile. The significance of differences in mean values (P) between compared parameters was evaluated using Student’s t-test (t).

RESULTS AND DISCUSSION

According to current literature, there is an increasing body of evidence indicating that pro-inflammatory mechanisms in cardiovascular diseases are mediated by

various cytokines, which can induce cardiomyocyte hypertrophy and apoptosis, fibrosis, and ultimately lead to adverse cardiac remodeling.

The obtained results are presented in Table 1 below.

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 [15].

Table 1.

Serum levels of pro-inflammatory cytokines in the examined children with CHDs

Parameter	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
Acyanotic CHD (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
Cyanotic CHD (with cyanosis), n=24				
IL-6	29,70±1,77***	29,55 [21,50; 37,82]	16,50	43,22
IL-8	51,77±1,45***	52,75 [47,11; 57,32]	38,91	62,30
TNF-α	65,76±3,27***	68,70 [50,45; 78,10]	39,44	89,72

Note: ** – significant compared with the control group (* – P<0.05, ** – P<0.01, *** – P<0.001). Me – median, Q1 (percentile) – 25%, Q3 (percentile) – 75%.*

Analysis of the obtained IL-6 results in the serum of children with acyanotic CHDs revealed statistically significant differences compared with the values of

healthy children in the control group. The serum IL-6 level in the acyanotic CHD group 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) (Fig. 1).

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 (CHDs). The obtained results suggest a possible link between IL-6 and CHDs, likely indicating activation of an inflammatory process associated with the heart defect, stimulation of cardiac tissue remodeling, involvement in the development of hypertrophy, and an imbalance in the immune system.

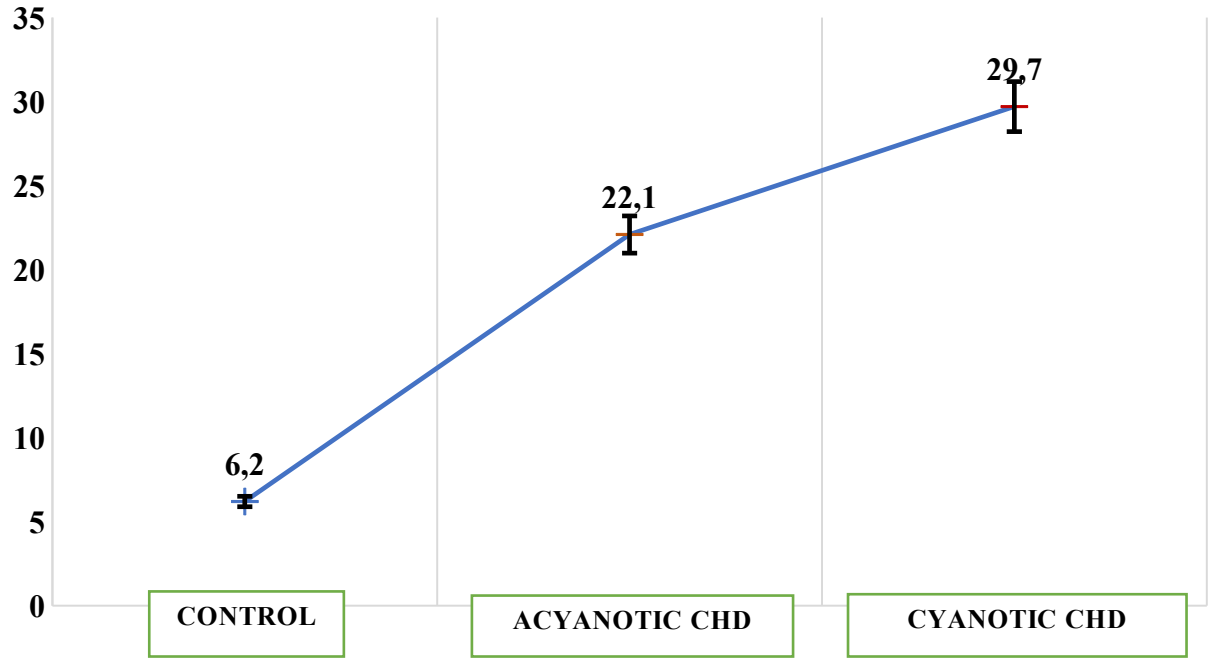

Fig. 1. Serum IL-6 levels in the examined children compared.

Note: * – significant compared with the control group (* – P<0.05, ** – P<0.01, *** – P<0.001)

Analysis of the data showed elevated IL-6 levels in the main group of children with cyanotic CHDs. The synthesis of this cytokine was increased 4.7-fold, with a mean value of 29.70 ± 1.77 pg/mL and an individual range of 16.5 to 43.22 pg/mL, compared to the control group, which had an average of 6.25 ± 0.43 pg/mL (P<0.001) (Fig. 1).

The elevated IL-6 levels in children with cyanotic CHDs may also indicate a strong inflammatory response associated with the heart defect and its consequences, as cyanosis is a condition in which insufficiently oxygenated blood circulates through the body, potentially causing stress and inflammatory reactions. IL-6 is one of the cytokines that can be produced in response to hypoxia and

hypoxia-induced tissue damage. Increased IL-6 levels may be related to immune system activation and inflammation in cardiac tissue and other organs affected by the heart defect, and may also serve as an indicator of the severity of the defect and its impact on the body.

As is well known, chemokines are a family of cytokines that induce directed leukocyte migration along a concentration gradient, leading to the accumulation of migrating cells at the source of chemokine production [13]. Therefore, the next stage of our research was to study the serum levels of IL-8, aiming to elucidate the role of this immune response mediator in CHDs.

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 the IL8 or CXCL8 gene [14].

To determine the role and involvement of this chemokine in normal conditions and in different types of CHDs, we examined serum IL-8 levels in our study as one of the key mediators of the immune response, serving as an activator of the angiogenic response.

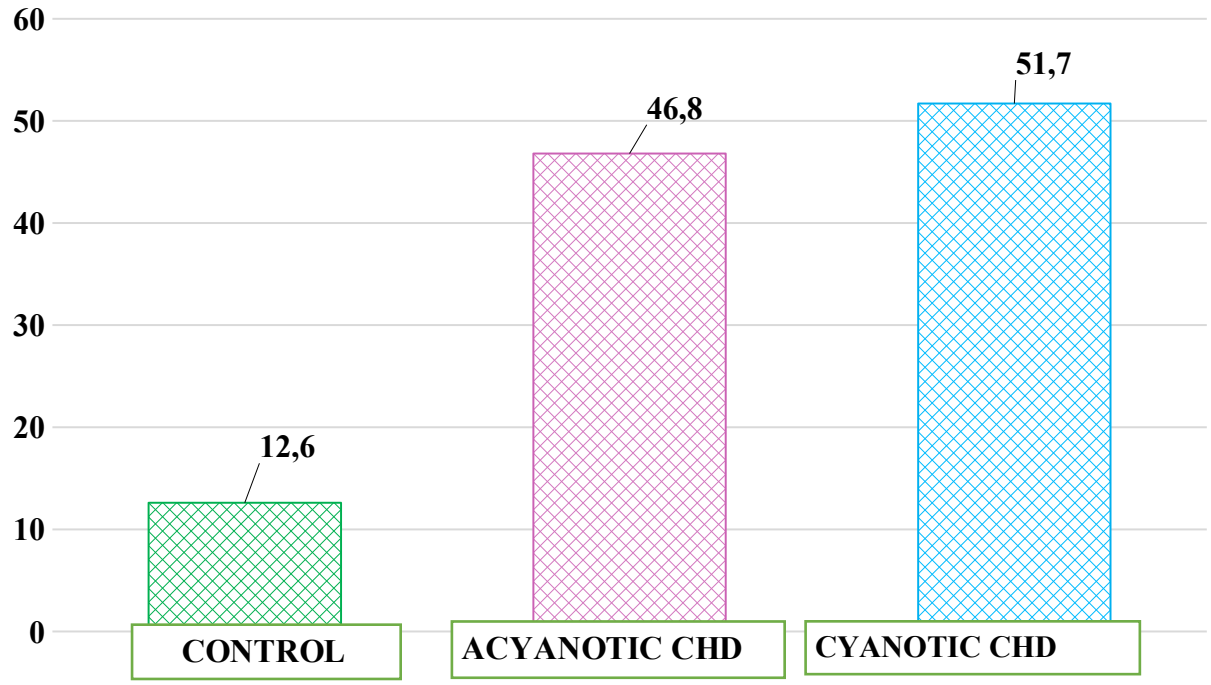

Fig. 2. Serum IL-8 concentration in the examined children compared.

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

Analysis of the obtained results revealed a relatively high IL-8 level in the group of children with acyanotic CHDs. The serum concentration of this chemokine in the main group of children 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, this value was 12.66 ± 0.60 pg/mL (P<0.001) (Fig. 2).

We hypothesize that the elevated interleukin-8 (IL-8) levels in congenital heart defects may be explained by several factors: 1) inflammation and immune activation, 2) angiogenesis, and 3) regulation of the immune response. Increased IL-8 levels may indicate an imbalance in the immune system, likely associated with acyanotic CHDs.

Assessment of serum levels of this chemokine in the group of children with cyanotic CHDs revealed significantly elevated values. The serum IL-8 level in children with cyanotic CHDs was increased 4.1-fold, with a mean value of 51.77 ± 1.45 pg/mL and a range of 38.9 to 62.3 pg/mL, compared to normative values of 12.66 ± 0.60 pg/mL (P<0.001) (Fig. 2).

In our opinion, the increased synthesis of interleukin-8 (IL-8) in children with cyanotic CHDs may be explained by several factors: 1) hypoxia induces the release of hypoxia-inducible factor (HIF-1α), which can activate the synthesis of IL-8 and other inflammatory cytokines; 2) abnormal blood circulation and structural heart anomalies can lead to tissue damage, triggering an inflammatory response and activation of immune cells, which may result in increased IL-8 synthesis, serving as a chemoattractant for neutrophils; 3) enhanced synthesis followed by neutrophil activation, as neutrophils can produce IL-8, amplifying the inflammatory response and further increasing IL-8 production around the affected area.

One of the key pro-inflammatory cytokines widely studied as a potential biomarker associated with heart failure is TNFα [12].

Tumor necrosis factor alpha (TNFα) is a member of the family of immunologically important proteins and a pro-inflammatory cytokine with a broad spectrum of activity. The main producers of TNFα 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 [10].

Comparative assessment of serum TNFα levels, shown in Fig. 3, revealed statistically significant results in the group of children with acyanotic CHDs. The synthesis of this cytokine in the group of children with acyanotic CHDs 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 the healthy children group, the mean value was 15.17 ± 0.49 pg/mL (P<0.001) (Fig. 3).

Elevated serum TNFα levels in the group of children with acyanotic CHDs likely indicate the development of cardiac cachexia syndrome in children with chronic congestive heart failure or chronic hypoxemia, and may reflect several factors: 1) TNF-α is an important mediator of inflammation and can be produced by immune cells in response to tissue damage; 2) TNF-α may be involved in remodeling processes and contribute to the development of fibrosis and hypertrophy. Increased TNF-α levels may reflect its involvement in pathological changes in cardiac tissue in congenital heart defects; 3) TNF-α is one of the key cytokines regulating the immune response, and its elevated levels may be associated with this imbalance.

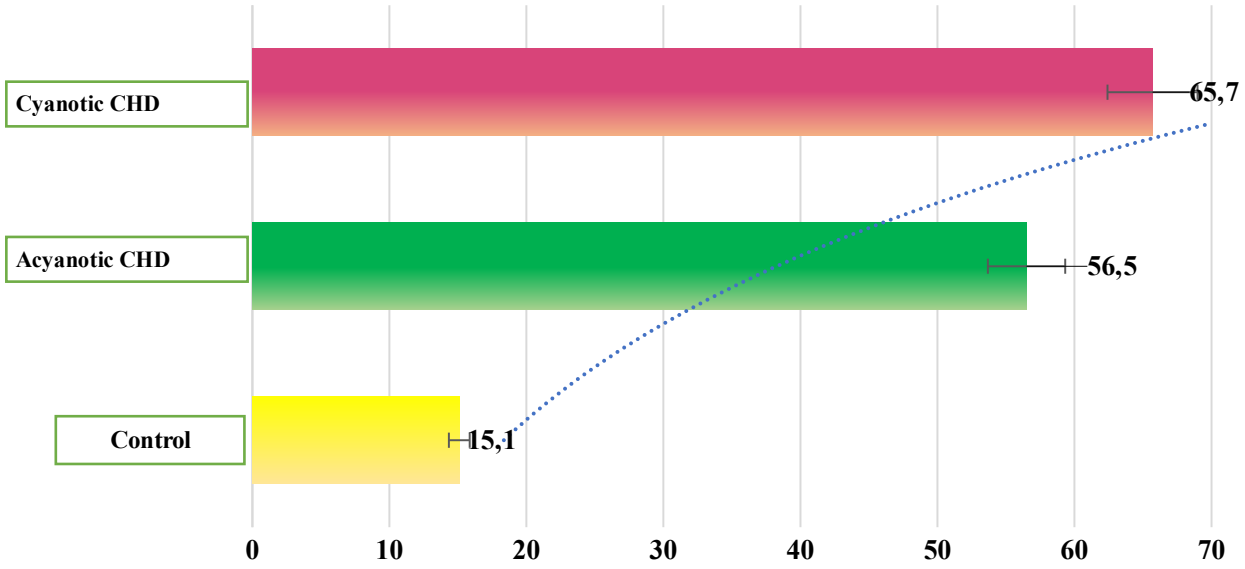

Fig. 3. Serum TNF-α levels in the examined children compared.

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

As shown in Fig. 3, the study of serum TNF-α levels in the group of children with cyanotic CHDs revealed a significant increase. The TNF-α level in the main group of children with cyanosis was elevated 4.3-fold, with a mean value of 65.76 ± 3.27 pg/mL compared to control values of 15.17 ± 0.49 pg/mL (P<0.001).

These results indicate that the increase in TNF-α levels in congenital heart defects may result from the interaction of several factors, such as inflammatory processes, tissue damage, and cardiac remodeling. These factors, in turn, may influence the progression of CHDs, which is associated with TNF-α and its direct damaging effects on cardiomyocytes, enhancement of oxidative stress, binding to membrane receptors, and involvement in the development of cachexia. Cyanotic CHDs can lead to impaired circulation and insufficient oxygen delivery to organs and tissues, potentially causing ischemia and hypoxia in the heart tissue and

surrounding areas. Ischemia and hypoxia contribute to immune system activation and inflammatory processes, which may result in increased TNF-α synthesis.

Thus, the study established that hypoxia and ischemia occurring in CHDs can stimulate increased production of various cytokines in response to tissue damage and stress. Congenital heart defects can trigger inflammatory responses in cardiac and vascular tissue due to abnormal blood flow and altered circulation. This may lead to elevated levels of various cytokines, such as IL-6, IL-8, and TNFα, which are key mediators of inflammation.

CONCLUSION

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 can lead to pathological changes and affect the development and progression of CHDs. Further research and clinical observations are needed to gain a more precise understanding of the role of these mediators in CHD pathology.

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