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CYTOKINE DIAGNOSTICS OF NEWBORNS BORN TO MOTHERS INFECTED WITH COVID-19

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ABSTRACT

The article is devoted to the development of a cytokine diagnostic method for predicting the development of critical conditions in newborns born to mothers with COVID-19, which is of great importance for health authorities when organizing specialized neonatology and pediatric services. Noninvasive urocytokinodiagnostics allows to achieve economic efficiency by reducing hospital beds, as well as the effectiveness of treatment due to minimal traumatization of newborns.

Keywords: *newborns, critical conditions, COVID-19, cytokines, immunity*

АННОТАЦИЯ

Статья посвящена разработке метода цитокиновой диагностики для прогнозирования развития критических состояний у новорожденных, рожденных от матерей с COVID-19, что имеет большое значение для органов здравоохранения при организации специализированной неонатологической и педиатрической службы. Неинвазивная уроцитокинодиагностика позволяет добиться экономической эффективности за счет сокращения больничных коек, а также эффективности лечения за счет минимальной травматизации новорожденных.

Ключевые слова: *новорожденные, критические состояния, COVID-19, цитокины, иммунитет.*

ANNOTATSIYA

Maqola ixtisoslashtirilgan neonatologik va pediatriya xizmatini tashkil etishda sog'liqni saqlash organlari uchun katta ahamiyatga ega bo'lgan COVID-19 bilan tug'ilgan yangi tug'ilgan chaqaloqlarda og'ir holatlar rivojlanishini bashorat qilish uchun sitokin diagnostika usulini ishlab chiqishga bag'ishlangan. Invaziv bo'lmagan urositokin diagnostikasi shifoxona yotoqlarining qisqarishi hisobiga iqtisodiy samaradorlikka, shuningdek, yangi tug'ilgan chaqaloqlarning minimal travmatizatsiyasi tufayli davolash samaradorligiga erishish imkonini beradi.

Kalit so'zlar: *yangi tug'ilgan chaqaloqlar, og'ir holatlar, COVID-19, sitokinlar, immunitet.*

INTRODUCTION

The frequency of critical diseases caused by COVID-19 is about 19%, some of which in most cases progress to acute respiratory distress syndrome and respiratory failure, accompanied by acute immune dysfunction.SARS-CoV-2 infection causes a sharp decrease in the number of lymphocytes, especially a decrease in CD4 T cells, accompanied by uncontrolled release of inflammatory cytokines, which leads to a second stroke and exacerbates pathological changes in the respiratory system. Clinical symptoms vary among the infected population, suggesting that individual immune status is associated with susceptibility to COVID-19 and that immune dysfunction may play a significant role in the development of critical diseases. Due to the special immunological status of pregnant women, the inflammatory reaction of the mother to a coronavirus infection can affect the structural and functional development of the fetus and newborn [2,5].

In children, COVID-19 is weak or asymptomatic.12; however, the virus can remain in the body for a long time, and viral nucleic acids can persist in feces, which suggests the possibility of non-respiratory transmission in children.Immaturity of immunological function in children and newborns leads to their increased susceptibility to viral infections, while immaturity of adaptive immunological development can make their clinical symptoms different from those in adults [1,3].

Together, these aspects raise serious questions about why the clinical manifestations of infected children and newborns differ from those of adults with immunosuppression and what effect the inflammatory response caused by maternal infection has on the immunological function of the fetus [4].

The purpose of the study:

Predicting the development of critical conditions in newborns born to a mother with COVID-19 by determining cytokines in the blood and urine.

Materials and methods of the study: The medical histories of 37 full-term and 22 premature newborns born to mothers with COVID-19 and hospitalized in inpatient treatment at the Department of Neonatology of the Bukhara Children's Multidisciplinary Medical Center in the periods from 2020 to May 2022 were retrospectively studied.

During their stay in the hospital, all patients were subjected to general clinical, laboratory, functional, biochemical, radiographic studies.

For a comparative assessment of the significance of cytokine status indicators in the prognosis of the development of critical conditions of newborns, a clinical and laboratory examination of 94 newborns was carried out: 33 newborns born from a mother with COVID-19 (group 1), 30 newborns with perinatal central nervous system (CNS), born from a mother with somatic diseases (group 2) and 31 healthy newborns born from a healthy mother.

Results:

As a result of the analysis of the cytokine content in the blood of newborns on the 2nd day of life, it was found that the concentrations of IL-17A in group 1 exceed the upper limit of the concentration range of these indicators in the group of healthy newborns (Table 1).

Table 1

The content of cytokines in the blood of newborns

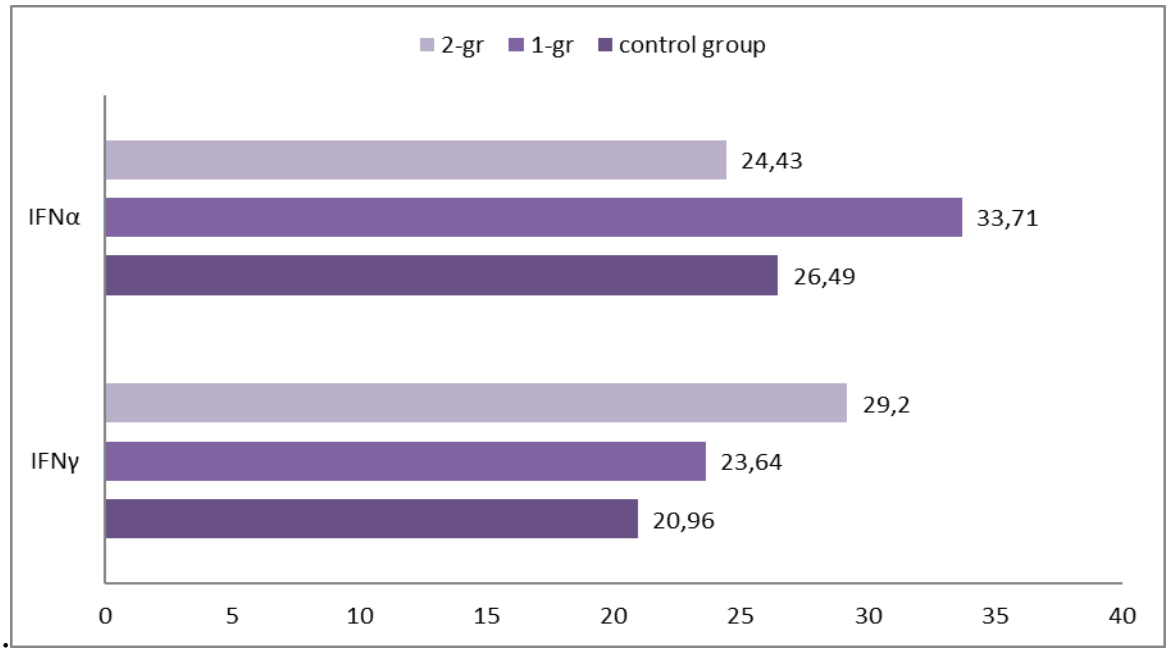
Cytokines pkg/ ml	Healthy newborns		1-group		2-group	
	min-max	average	min-max	Average	min-max	average
IFNγ	14,48 -27,35	20,96±0,66	15,27 - 32,25	23,64±0,81*	17,05- 39,63	29,20±1,28*
IFNα	15,83- 38,21	26,49± 1,20	21,79- 47,37	33,71± 1,22*	15,83- 38,21	24,43± 1,36
IL-17A	29,93- 64,97	46,99± 1,70	55,34- 92,06	69,68± 1,70*	24,22- 56,17	38,74± 2,07*
MCP-1	98,29- 305,71	196,69± 9,92	422,15- 1058,15	765,66± 33,07**	98,29- 305,71	116,47± 7,86*
VEGF	19,21- 59,93	38,47±2,23	25,17-54,67	40,05±1,49	19,21-59,93	42,15±1,82

Note:*-significantly relative to the healthy group (*-p<0.05, * p<0.01)

The interferon status of newborns of the 1st and 2nd groups is characterized by a significant increase in IFNy to 23.64±0.81 and 29.20±1.28 pkg/ml, respectively, against the indicators of the control group-20.96±0.66 pkg/ml (-p<0.05).

At the same time, IFNa has a statistically significant tendency to increase in newborns of the 1st groups-33.71± 1.22 pkg/ml (p<0.05), with respect to the indicators of the control group-26.49± 1.20 pkg/ml.

And in newborns of the 2nd group, its value was at the level of control indicators



(Fig.5).

Figure 5. Interferon status of newborns in the first day of life

The most studied representative of the IL-17 family is the cytokine IL-17A. This cytokine plays a central role in inflammatory processes, so the regulation of its functioning is of great importance for the body. Numerous mechanisms of signaling pathways of such regulation are known, presumably neutralizing the side effects of inflammation. IL-17 plays a key role in protecting the body from extracellular bacterial and fungal infections [2].

At the same time, the key cytokine IL 17A, which determines the lesion of bone tissue, plays a complex role in this process. It has been shown that IL 17A causes bone resorption in experimental arthritis. This effect is associated with the activation of the RANKL system, as a result of which the activity of osteoblasts is inhibited, therefore, the production of bone tissue decreases [4].

In our studies, IL-17A in group 1 newborns was increased to 69.68± 1.70 pkg/ml, compared to the control group -46.99± 1.70 pkg/ml ($p<0.05$), and in group 2 it has a significant tendency to decrease to-38.74± 2.07 pkg/ml ($p<0.05$), against the control values of -46.99± 1.70 pkg/ml.

Over the past decade, it has been shown that monocytic chemoattractant protein-1 (MCP-1) and vascular endothelial growth factor (FRF) can be considered one of the leading molecular markers of vascular endothelial damage. MCP-1, the most specific for monocytes, belongs to the class of chemokines [5].

One of the main chemokines for monocytes/macrophages and activated T-lymphocytes is monocytic chemotactic protein-1 (MCP-1). MCP-1 was first

identified as a product of secretion of monocytic leukemic cells stimulated by lipopolysaccharide, as well as peripheral blood mononuclear cells. MSP-1 belongs to the class of CC chemokines and is a powerful chemoattractant of monocytes/macrophages. MCP-1 is not only a chemoattractant that ensures the migration and extravasation of mononuclear cells into the focus of inflammation, but also a mediator of inflammation, activating resident cells at the same time. Human MCP-1 is a protein consisting of 76 amino acids. MCP-1 is produced by many types of cells, including mononuclear cells, mast cells, T cells, osteoblasts, fibroblasts, endothelial cells, bone marrow cells, epithelial cells, astrocytes. The synthesis of MSR-1 is induced by IL-1ß, α-TNF, γ-INF, IL-6, IL-4. Under the influence of MSP-1, proliferation of vascular smooth muscle cells also occurs with their secretion of proinflammatory cytokines that contribute to the progression of the disease due to vascular damage [1].

As a result, a 3.89-fold increase in MSR-1 was found in group 1 newborns-765.66± 33.07 pkg/ml, against the control - 196.69± 9.92 pkg/ml. In group 2 newborns, a statistically significant decrease in MSR-1 was found to 116.47± 7.86 pkg/ml, against the control - 196.69± 9.92 pkg/ml.

The study of another vascular endothelial growth factor, VEGF, showed that there was no connection between its synthesis and the development of critical conditions in newborns on the first day of life.

Consequently, in the presence of a coronavirus infection in the mother, on the first day of life, the child begins to activate the proliferation of vascular smooth muscle cells with the secretion of proinflammatory cytokines.

According to the research results of V.V.Nikitin and co-author (2010), as the disease progresses, the risk of vascular damage increases, the synthesis of cytokines increases, including IFNy. The results of the studies showed a significant increase in the synthesis of IFNy in the first day of life in newborns, regardless of the presence of somatic and infectious diseases of the mother. At the same time, IL-17A protects the mother's body from extracellular bacterial and fungal infections, thereby resorption of bone. On the other hand, osteoporosis is promoted by the use and use of anticoagulant drugs in the treatment of COVID-19.

Consequently, an increase in the concentration of IL-17A by 1.48 times in the blood of newborns born from a mother with COVID-19 shows the risk of developing both infection and osteogenesis disorders.

In order to assess the dynamics of cytokine synthesis, the above cytokines were studied in the urine of newborns of the examined groups on the 7th day of life. As a result, an increase in IFNy was found to be 2.84 times in group 1 newborns, 1.72

times in group 2 newborns ($p<0.001$), against the control -5.94±0.23 pkg/ml ($p<0.05$). With respect to IFNa, an increase to 7.60± 0.39 pkg/ml was also detected in urine. in group 1 newborns, up to 9.49± 0.43 pkg/ml in group 2 newborns, the indicators of the control group were 5.78± 0.23 pkg/ml, the results obtained were reliable in the range of $p<0.05$ (Fig.7., Table 2

Table 2.

The content of cytokines in the urine of newborns

Cytokines pkg/ml	Healthy newborns		1-group		2-group	
	min-max	average	min-max		min-max	average
IFNγ	4,25-8,75	5,94±0,23	10,08- 24,11	16,84±0,66***	6,94- 15,42	10,21±0,41*
IFNα	3,68- 8,06	5,78± 0,23	4,33- 11,87	7,60± 0,39*	5,48- 14,33	9,49± 0,43*
IL-17A	20,05- 38,48	30,75± 0,93	41,15- 92,50	65,48± 2,3**	23,55- 54,67	37,07± 1,43*
MCP-1	74,51- 130,20	99,25± 2,63	16,72- 33,10	23,36± 0,75***	39,45- 71,27	54,75± 1,8**
VEGF	18,36- 34,97	26,99±0,87	10,26-30,15	19,79±1,02*	14,48-33,05	22,72±0,96*

Note:*-significantly in relation to the healthy group ($p<0.05$)

At the same time, there is an increase in the level of IL-17A in the urine of group 1 newborns by 2.2 times (65.48± 2.3 pkg/ml), up to 37.07± 1.43 pkg/ml in group 2 newborns against control values of -30.75± 0.93pkg / ml ($p<0.05$).

According to the literature, it is known that the endothelium of the intima of the vessels performs barrier, secretory, hemostatic, vasotonic functions, plays an important role in the processes of inflammation and remodeling of the vascular wall.

In contrast to the indicators of VEGF in the blood, in urine studies VEGF compared to the control -26.99±0.87pkg/ml, was reduced to 19.79±1.02 pkg/ml and 22.72±0.96 pkg/ml in newborns of the 1st and 2nd groups, respectively ($p<0.05$). All the obtained results of the study of cytokines in the urine of newborns had statistical significance in the ranges from $p<0.05$ to $p<0.0001$.

Thus, the obtained results of the study of cytokines in urine show activation of interferon status by the 7th day of life against the background of an increase in the key cytokine of bone resorption (-IL-17A). At the same time, there is a decrease in urine of the leading molecular markers of vascular endothelial damage - MSR-1 by 4.25 times and 1.82 times at birth of children from a mother infected with COVID-19 (group 1) and with other somatic diseases (group 2), respectively. And VEGF also decreases significantly in newborns, both with COVID-19 infection in the mother and in its absence. When comparing the results of the cytokine status with clinical and biochemical data, symptoms of systemic inflammation are noted in parallel on day 7 in patients with group 1 newborns: an increase in body temperature, leukocytosis, tachycardia, an increase in reactive protein and a change in the prothrombin index.

Some differences in the values of this indicator in the blood and urine of group 2 newborns have been established. Thus, with an increase in IL-17A and MCP-1 in the blood on the first day of life, VEGF tends to increase to 42.15 ±1.82 pg/ml, which shows the risk of developing a systemic inflammatory response syndrome at the level of blood vessels with endothelial damage. On the 7th day of life, there was a tendency to increase IL-17A in urine to 37.07± 1.43 pkg/ml, than in the healthy group-30.75± 0.93 pkg/ml $p<0.05$.

The obtained results of the study show the accumulation of cytokines in the focus of inflammation and indicate the activity of the inflammatory process, requiring correct anti-inflammatory therapy.

Thus, the advantages of noninvasive immunodiagnostics in neonatology have been established by the determination of cytokines in the urine of newborns. The control of urine cytokines in dynamics determines the prognosis of the development of critical conditions in newborns both early and in the late period of adaptation.

CONCLUSION

In newborns born to a mother with COVID-19, an increase in IFNy and IFNa in the blood is noted on the first day of life against the background of an increase in IL-17A by 1.48 times, which shows the risk of developing both infection and osteogenesis disorders. Activation of interferon status by the 7th day of life in newborns was established against the background of an increase in the key cytokine of bone resorption (-IL-17A). A decrease in the urine of molecular markers of vascular endothelial damage- MSR-1 by 4.25 times was found in newborn children born from a mother infected with COVID-19. A decrease in VEGF in the urine of newborns was found, both with COVID-19 infection in the mother and in its absence.

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