CYTOKINE PROFILE IN NEWBORN CHILDREN WITH INTRAUTERINE INFECTIONS

The study examines production of proinflammatory IL-1b, IL-8, γ-IFN and anti-inflammatory (IL-4) cytokines in newborns from mothers with healthy pregnancy and with the intrauterine infections. Under supervision there were 55 children, newborn from the mothers with herpes virus infections: from them 21 in the early neonatal period, 19 in the late neonatal period. The control group included 15 healthy children newborn from healthy mothers with favourable course of pregnancy.

The analysis has shown that γ-IFN level, in the early neonatal period in children with intrauterine infection, was in the average 7.0±0.73 pg/ml or 3.6 times lower (P<0.05) than control. In the late neonatal period production of γ-IFN increased up to 11.2±0.74 pg/ml (P<0.05), but without reaching of control meanings. The reduced production of γ-IFN results, apparently, to long recurrent disease.

The performed examinations have shown that the development of pathological process at herpes virus infection is accompanied by significant balance disturbance of proinflammatory and anti-inflammatory cytokines during all neonatal period. The revealed cytokine profile changes in the newborns in the short age periods and their severity degree show important pathogenic role of immune mechanisms in the development and progressing of herpes virus infections.

Keywords: Cytokines, interleukins, interferon.

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The intrauterine herpes virus infection in the current perinatology presents a significant problem (Kadcina, 2007; Kudashov, 2006; Hoppen et al., 2001; Tremolada et al., 2008). Despite the successes associated with the development and introduction of new informative methods of diagnosis, there is a significant number of women who had no medical examinations, which resulted in high percentage of perinatal morbidity and mortality (Nisevich et al., 2009; Mamedbeili and Ragimova, 2009).

In Uzbekistan, in the structure of early neonatal mortality, in accordance with the recommended by WHO international criteria of live births, the infectious diseases occupy the fourth place (Umarova et al., 2009). However, in the medical statistical documentation of the republic there is no special registration of intrauterine infections.

Damaging effect of viruses on the immunocompetent cells, as well as their long persistence in various organs and tissues, causes a wide spectrum of pathology both in the neonatal period and in older age. Besides, it is known, that according to teratogenic significance CMV infection occupies the second place after rubella (Leshkevich et al., 2008; Orekhov et al., 2004). Intrauterine development of the child occurs under conditions of complex immunological interrelations between mother and fetus. The formation of immune status is connected mainly to the state of mother’s health and development of pregnancy. The functional leukocyte immaturity, insufficiency of cellular cooperation in formation of immune response, immunosuppressive factors during gestation predict high predisposition of the newborn to infection (Gankovskaya et al., 2007; Zdravkovic et al., 1997). The study of immune and interferon status of the pregnant women suffering from infectious diseases, undoubtedly, has a great importance. It is established, that in neonates born to mothers with the focuses of infection, the interferon-producing leukocyte ability is reliably attenuated (Samsigina et al., 1997). According to the data of Budanov et al. (2009) the presence of intrauterine infection, confirmed by
bacteriological and virology investigations of amniotic waters, as well as postnatal, production of spontaneous interferon (IFN)-alpha increased in mother’s venous blood. And in these cases is noted 2 times increase in serum IFN-alpha, the production of IFN-gamma considerably increased as well. The revealing of changes of blood cytokine status in mother allows to predict risk of intrauterine infection development of an infection in fetus in 93.3% of cases. In the neonatal period during maturation of anti-infectious defense system of the body the gamma-chain of interferon system has become of great importance. However, the synthesis of interferon is reduced, particularly in very premature infants (Malinovskaya, 1999).

The literature reports indicate the activation of cells of monocytic-macrophage line before the birth of a child, the prevalence of influence of proinflammatory cytokines in the umbilical blood of the healthy neonates in comparison with adults (Bessler et al., 1999). This is accompanied by physiological leucocytosis and neutrophilosis. The study of IL-6 and IL-8 concentration in the serum of umbilical blood has revealed, that in the majority of healthy newborns these levels are very small and do not exceed a threshold of sensitivity of used immunoenzymatic systems. Volodin with coauthors showed that to the 3-7 day of neonate life the plasma levels of proinflammatory and anti-inflammatory cytokines became higher (Volodin et al., 2000). Within the first week of life in the physiological conditions there is reliable increase of plasma levels IL-1b, which can be explained by activation of the cells of monocyte-macrophage origin as a response to the effect of the great number of exogenous antigens (bacteria, viruses) at the early neonatal period of adaptation. But in the physiological conditions, despite the increase of quantity of the proinflammatory mediators there is no clinical systemic inflammation, and newborns are discharged in satisfactory condition by the end of the first week of life.

The development of the system of inflammatory response, evidently, prevents the increase in the synthesis of proinflammatory cytokine IL-4 by lymphocytes. The increase in the level of IL-4 production is accompanied by reduction of absolute number of peripheral blood of newborns. In the early period of postnatal immune adaptation of healthy full-term newborns during high antigen loading, the supporting of immune homeostasis in the newborn body is provided by the whole complex of immunological mechanisms major among which is the activation, proliferation and differentiation of the producer cells. However, in neonates with intrauterine infections have been found that changing the relationship between proinflammatory cytokines, the most sensitive and specific parameter is the IL-8 (Volodin et al., 2000).

Cytokines as universal mediators realize interaction of immune system with other systems of the body ensuring preservation of homeostasis. In diseases induced by herpes viruses (SHV and CMVI) the “paradox” of constant immune response against a latent infection takes place. The activation of herpes viral infection always occurs on the basis of disturbances in the cytokine profile. The activation of herpes viral infection always occurs during disturbances in the cytokine profile. Herpes viral infection stimulates secretion of the whole number of cytokines with different cells - producers. Thus, cytokines carry out various roles in occurrence and course of infection: cytokines of Th1-type promote reduction of replication and acceleration of clinical recovery, while cytokines of Th2-type have the opposite effect.

In this connection, the study of cytokine profile in neonates with intrauterine infections during all neonatal period is of great interest to us. The purpose of the present investigation was to study the state of cytokine profile in newborns with herpes viral infections in early and late neonatal periods.

**Material and methods**

Totally there were studied 55 newborn children, who were born by mothers with herpes virus infections: of them 21 newborns in early neonatal period, 19 newborns - in the late neonatal period, and group of included healthy newborns - 15, who was born to
practically healthy mothers with favorable course of pregnancy and from physiological labor.

Among the total number of neonates born from the mothers with herpes virus infection there were 11 (27.5%), from the mothers with associated herpes virus and cytomegalovirus (CMV) -22 (55%). The other 8 (17.5%) additionally to the above specified infections were infected with the third one (chlamydiosis, ureaplasmosis, mycoplasmosis). The study of neonates was performed in the Scientific Research Institute of Obstetrics and Gynecology and Department of neonatal pathology in the urban children's hospital №5. The identification of the diagnosis was performed with the use of immune enzymatic analysis (IEA) for the presence of specific antibodies and polymerase chain reaction (PCR)

Was studied the production of proinflammatory (IL-1, IL-8 and γ-IFN) and anti-inflammatory (IL-4) cytokines in newborns in norm and with intrauterine infections. The principle of functioning of test - system for definition of cytokines (developed by State Scientific Research Institute S.-Petersburg, Closed Joint-Stock Company “Protein Contour” and “Cytokine”) is based on a “sandwich”-method of Solid-Phase Immune-enzymatic analysis with the use of horse-radish peroxidase enzyme indicator. Quantitative evaluation of the results was made by the method of construction of calibrated curve or with the use of the commercial computer program “Microplate manager”, reflecting the dependence of optical density on concentration of a standard antigen and allowing the comparison with the studied samples. Sensitivity of the method is 5-30 pg/ml.

**Results and discussion**

The study of obstetric anamnesis in the surveyed women showed spontaneous abortion in 9 (22.5%), medical abortions - in 6 (15%), still-birth - in 5 (12.5%), not developing pregnancy - in 3 (7.5%) cases. Antenatal development of newborns was complicated by an aggravation of the focuses of chronic infection in the mother in 31 (77.5%), anemia - in 23 (57.5%), ARVI - in 24 (60%), toxicoses - in 13 (32.5%), threat of interruption of pregnancy and gestosis in 30 (75.5%) newborns.

During analysis of intrauterine infection clinical picture was revealed multiple lesions of various organs and systems. The most frequent symptoms were intensive jaundice (72.5%), hepatosplenomegaly (42.5%), anemia (65%). In the significant part of children (45%) were observed signs of intrauterine pneumonia.

The impairment of the central nervous system was noted in the overwhelming majority of newborns and was expressed as syndrome of depression in 52.5%, syndrome of hyperexcitability in 22.2%, in 2 (5%) - convulsive syndrome, in 5 (12.5%) - hypertensive syndrome. In two children developed brain edema, in 3 (7.5%) - progressing hydrocephalia. Special attention was paid to the fact of birth of three children with congenital defects of development, in one of them the multiple defects of development were found, in the second one - congenital heart disease, and in the third one - on neurosonography was found total atrophy of substance of the left brain hemisphere. Thus, the investigations performed in newborns, have revealed multifarious clinical manifestations of perinatal infections in the neonatal period.

To understand the pathogenic mechanisms occurring in intrauterine infections in newborns we studied the features of balance disturbance of proinflammatory and anti-inflammatory cytokines, in many respects determining a clinical condition of the child.

We carried out investigation to determine the levels of IL-1β production as the important mediator which is one of the most universal regulators of immunity and inflammatory reactions with a wide spectrum of biological effects, including proliferation of T- and B-lymphocytes, antibody formation, induction of other cytokine synthesis, etc. In early neonatal period the newborns with intrauterine infection had reliably increased (4,7 times) the level of IL-1β production in comparison with healthy newborns (948.96±32.63 pg/ml against 200.5±11.1 pg/ml, P<0.001). In the late neonatal period the synthesis of IL-1β
increased 7 times and reaches the level 1405.8 ±77.02 pg/ml against 200.5+11.1 pg/ml (P<0.001) (Table 1.). The data received allow thinking of presence of the certain dependence of the level of IL-1β production on the character of pathological process.

| TABLE 1. CYTOKINE CONCENTRATION IN NEWBORNS WITH INTRAUTERINE INFECTIONS |
|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                               | Control (C) | Early neonatal period (ENP) | Late neonatal period (LNP) | P1    | P2    | P3    |
|                               | n=15 (1-10) | (1-5) n=21              | (7-10) n=19              | C-ENP | C-LNP | ENP-LNP |
| IL-1β                         | 200.5±11.1   | 948.96±32.63            | 1405.8±77.02            | P<0.05| P<0.05| P<0.05 |
| IL-4                          | 60.1±4.43    | 20.7±1.76               | 23.3±2.14               | P<0.05| P<0.05| P>0.05 |
| IL-8                          | 181.3±7.42   | 486.33±34.20            | 702.4±41.47            | P<0.05| P<0.05| P<0.05 |
| γ-IFN                         | 25.2±1.94    | 7.0±0.73                | 11.2±0.74              | P<0.05| P<0.05| P<0.05 |

Activation of specific immune response is related to some cytokines (IL-2, IL-4 etc.), regulating growth and differentiation of lymphocytes and contributing to this phase of immune response. Of them IL-4 has a wide spectrum of biological action, the most known is inhibition of inflammatory response and increase in humoral immunity, inhibition of monocytary-macrophagal system by blocking the activating effects of γ-IFN, and helping to curb the activity of NK, macrophage and activation of T-suppressors.

In practically healthy newborns the level of IL-4 was, on the average, 60.1±4.43 pg/ml, and in early neonatal period is observed a decrease of its level up to 20.7±1.76 pg/ml, that is reliably lower, than in the control (P<0.05). In late neonatal period the concentration of IL-4 remaining reliably low (23.3±2.14 pg/ml) in relation to the control parameters, has tendency to increase, but it does not differ from the parameter of early neonatal period.

It is known, that IL-8 induces hemotaxis in T-lymphocytes, stimulates differentiation of T-helpers, growth and activity of T-lymphocytes and cell-killers. In the serum of peripheral blood of healthy neonates the level of IL-8 was, on the average, 181.3±7.42 pg/ml, and in early neonatal period the synthesis of IL-8 raised 2.7 times and became, on the average, 486.3±34.20 pg/ml (P<0.05). In the late neonatal period the concentration of IL-8 raise 3.9 times and reaches up to the level of 702.4±41.47 pg/ml, thus it remains reliably higher than the parameter of early neonatal period (P<0.05).

The important role in coordination of functional associations of multicomponent immune system belongs to interferons, presenting a group of biologically active proteins or glucoproteides, synthesized by a cell during protective reaction to foreign antigens. From them γ-IFN is generated owing to T-lymphocyte stimulation and NK and is produced only by immunocompetent cells. It also plays an important role in the development of the immune response to Th1-type, promoting expression of β2-subunits of the receptors for IL-12. Besides, γ-IFN blocks the development of Th2-type, inhibiting IL-4 synthesis and cell proliferation of Th2- phenotype. It has no antiproliferative effect on cell of Th1-phenotype, because they do not express β2-subunit of γ-IFN receptor.

The analysis of the results of study of γ–IFN level in the peripheral blood serum in healthy newborns has shown that its concentration was 25.2±1.94 pg/ml, while in the early neonatal period in children with intrauterine infection the level of γ-IFN was, on the average, 7.0±0.73 pg/ml, which was 3.6 times lower (P<0.05) than the control one. In the late neonatal period production of γ-IFN increased up to 11.2±0.74 pg/ml (P<0.05), but without reaching the control meanings. The reduced production of γ-IFN results, apparently, in long recurrent disease.

Thus, the investigations performed have shown that the development of pathological process in herpes virus infection is accompanied by significant balance disturbance of proinflammatory and anti-inflammatory cytokines during all neonatal period. The revealed cytokine profile changes in the newborns in the short age periods and the degree of their
severity show the important pathogenic role of immune mechanisms in the development and progression of herpes virus infections.

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