IMMUNOLOGICAL FEATURES OF UMBILICAL CORD BLOOD OF NEWBORNS BORN TO WOMEN WITH DIFFERENT IMPLEMENTATION OF INTRAUTERINE INFECTION

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Perinatal infections (PNIs) have been increasingly referred to as one of the causes of adverse effects of pregnancy recently, accompanied by an inflammatory process, which is based on a highly integrated immune response. Any infectious disease, regardless of the nature of the pathogen, is accompanied by activation of the immune system [1,2].

According to the literature, the state of resistance to infection is formed by numerous reactions of the immune system, the main function of which is to recognize and eliminate infectious agents, as well as the products of their activities [3,4,5,6]. Under the influence of infectious agents there are changes in the immune system that contribute to the chronicity of the infection and the development of secondary immune deficiency in pregnant women. Immunopathological reactions cause destabilization of the whole homeostasis and create a favorable basis for the periodic exacerbation of infectious and inflammatory diseases, and the inability of the pregnant woman to adequate immune response is the cause of pathological pregnancy and the possibility of abortion at any time [7,8]. This category of pregnant women is distinguished into a risk group for the development of IUI, which is one of the leading causes of perinatal morbidity and mortality [9,10].

Determination of immunological parameters in umbilical blood reflects the degree of formation of the immune system of the fetus and thus characterizes the level of anti-infective protection, which depends on the risk of infectious abnormalities [11].

Objective: To investigate the immunological features of umbilical cord blood of newborns born to women with different implementation of intrauterine infection (IUI).

Materials and methods. The study involved examination of 180 pregnant women divided into 3 groups depending on the presence and nature of the detected infection: Group I - viral (CMV, herpes simplex virus types1,2,6), Group II - bacterial (chlamydia, ureaplasma, mycoplasma) and Group III - mixed (viral and bacterial) infection. Each of these groups was divided into 2 subgroups: with the subsequent implementation of the infection in newborns (1) and without it (0). This group was the main one in the study. The control group included 50 patients with physiological pregnancy.

In clinical trials, samples of umbilical blood obtained during childbirth from women with signs of IUI after the use of different treatment regimens depending on the etiological factor of PNI were studied.

Evaluation of infectious status was performed by serological methods using enzyme-linked immunosorbent assay (ELISA) to determine the concentration of anti-infective IgM, IgG and IgG avidity index to *Chlamydia trachomatis, Ureaplasma urealiticum, Mycoplasma hominis*, and herpes simplex viruses types 1-2 (HSV), herpes virus type 6 (HSV-6) and cytomegalovirus (CMV).

Determination of the subpopulation composition of umbilical cord blood lymphocytes was performed on a flow cytometer FACSCalibur (USA) (CellQuest Pro software) using standard protocols.

The obtained data were subjected to statistical processing using STATISTICA software. Kolmogorov-Smirnov consistency criterion was used to check the coincidence of the distribution of quantitative indicators with the normal in groups. As the law of distribution of the numerical indicators under investigation differed from normal, the study involved methods of nonparametric statistics. The Kraskel-Wallis test (to check the dependence of the indicator on the subgroup) and the Mann-Whitney test (to compare the indicators of each subgroup with the control group) were used sequentially to assess differences with the control group. The Mann-Whitney test was used to identify statistically significant differences between subgroups within each of the three main groups.

Results and discussion. Phenotyping of lymphocytes showed that in groups with the implementation of IUI, regardless of the etiological factor, the average total number of T-lymphocytes ($CD3^+$) was lower by 27.15-34.89% of the values obtained in the examination of umbilical blood samples of comparison groups (Table 1). The maximum number of $CD3^+$T-lymphocytes, which was 21.8% higher than the control values, was found in the group of newborns I-0. The lowest total number of $CD3^+$T lymphocytes was in Group III-1, which is 38.4% lower than the reference values.

This trend was registered for the main subpopulations of T lymphocytes: in the groups with the implementation of IUI the level of $CD4^+$T lymphocytes was significantly lower than in similar comparison groups. The percentage of cytotoxic $CD8^+$ lymphocytes was also higher in the groups with a favorable course of IUI (Table 1). As a result, the values of the immunoregulatory index in these groups were higher than in the main groups with the implementation of IUI.

Table 1. Phenotypes of T-lymphocytes of umbilical blood of newborns

Clinical groups	Indicators			
	Total T-lymphocytes $CD3^+$, %	T-helpers $CD4^+$, %	T-suppressors $CD8^+$, %	IPI CD4/CD8
Control	56.2 (44.0–66.0)	29.8 (21.5–39.0)	22.3 (13.0–29.5)	1.9 (0.8–2.3)

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I-1	44.6*/** (32.0–51.5)	24.1 (19.0–26.5)	16.4 (11.0–19.5)	1.46** (0.76–1.9)
I-0	68.5* (54.5–70.0)	36.5* (28.0–46.5)	19.5 (11.0–24.5)	1.8 (1.1–2.2)
II-1	41.6*/** (28.0–56.0)	29.0** (21.0–38.0)	12.9** (11.0–17.5)	2.2 (1.9–2.5)
II-0	62.2 (48.0–69.5)	46.0* (39.5–51.0)	18.6 (13.0–27.5)	2.4 (1.4–2.7)
III-1	34.6*/** (22.0–42.5)	26.6** (19.0–31.5)	15.1** (10.0–18.0)	1.7 (1.0–2.1)
III-0	47.5 (30.5–61.0)	49.8* (46.3–51.0)	21.4 (18.0–28.5)	2.3 (1.8–2.7)

Note: * the difference is statistically significant compared to the control,

** the difference is statistically significant in subgroups (Kraskel-Wallis test, Mann-Whitney test, p <0.05).

Assessment of natural $CD16^+$ killer cells level showed an increased number relative to control in all groups, but in subgroups with the implementation of IUI content of NK cells was higher by 16.4% -59.8% than in comparison groups (Table 2).

The concentration of antibody-producing B-lymphocytes did not differ statistically in groups with IUI of bacterial and combined etiology, regardless of its consequences, but in Group I-0 the content of B-lymphocytes was 21.3% higher than their level in Group I-1.

The activity of regulatory T-lymphocytes expressing $CD25^+$ differentiation cluster in the groups with favorable IUI effects was higher relative to control values and relative to similar subgroups with IUI implementation. In the group with the development of viral infection, the activity of regulatory cells was the lowest, while in the group with the implementation of bacterial and combined IUI, the number of $CD25^+$ T-lymphocytes did not differ significantly from the control (Table 2).

Table 2. Phenotypes of immunocompetent cells of umbilical blood of newborns

Clinical groups	Indicators		
	Natural killers, $CD16^+$, %	B-lymphocytes, $CD19^+$, %	Regulatory T-lymphocytes $CD25^+$, %
Control	8.7 (6.0–12.0)	12.5 (11.5–19.0)	25.6 (18.6–32.0)
I-1	17.5** (12.6–22.8)	13.6** (12.0–15.5)	21.6*/** (17.2–24.6)
I-0	13.0 (9.0–15.0)	16.5* (11.5–18.0)	29.3 (22.6–32.4)
II-1	17.0** (14.5–25.6)	12.0 (9.0–15.0)	24.7** (22.0–29.5)
II-0	14.6* (6.0–18.6)	11.2 (9.0–16.5)	30.4* (26.0–39.4)
III-1	18.2** (9.8–24.6)	13.9 (8.5–17.3)	23.4** (17.5–27.4)
III-0	11.6 (6.5–13.9)	14.3 (10.2–16.4)	36.8* (24.0–42.5)

Note: * the difference is statistically significant compared to the control,

** the difference is statistically significant in subgroups (Kraskel-Wallis test, Mann-Whitney test, p <0.05).

The study of humoral parameters of umbilical blood immunity in all newborns revealed a low concentration of IgA, which in clinical groups was slightly higher than control, but did not differ statistically in subgroups with different consequences of IUI. The level of IgM in the groups with implementation of IUI was statistically lower than in the comparison groups, but with all the consequences of the infectious process, the concentration of IgM significantly exceeded the control values. The content of IgG in umbilical serum in the groups of newborns did not exceed the range of reference values, but in the subgroups with clinical manifestations of IUI IgG concentration was lower by 13–17% than in similar comparison subgroups (Table 3).

Table 3. Concentration of immunoglobulins in umbilical blood of newborns

Study groups	Indicators		
	IgA, g/l	IgM, g/l	IgG, g/l
Control	0.01 (0–0.03)	0.20 (0–0.22)	12.4 (4.5–18.8)
I-1	0.09* (0.03–0.15)	0.32*/** (0.1–0.38)	11.2 (6.0–12.8)
I-0	0.08* (0.03–0.13)	0.40* (0.20–0.45)	13.2 (9.8–15.0)

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II-1	0.13* (0.08–0.17)	0.56*/** (0–0.6)	12.1 (7.6–14.3)
II-0	0.12* (0.09–0.14)	0.80* (0.2–0.96)	14.6 (8.8–16.6)
III-1	0.05* (0–0.09)	0.36*/** (0.11–0.40)	11.9 (5.6–16.0)
III-0	0.06* (0.02–0.10)	0.83* (0.34–1.1)	13.7 (8.0–14.8)

Note: * the difference is statistically significant compared to the control, ** the difference is statistically significant in subgroups (Kraskel-Wallis test, Mann-Whitney test, $p < 0.05$).

Phagocytic activity of umbilical blood neutrophils in neonates of the control group ranged in a wide range of values. The number of cells actively involved in antigen uptake ranged from 26% to 70%. In groups with the presence of PNI of any etiology without its implementation, the average value of the phagocytic index ranged from 51% to 56%, but in groups with the implementation of IUI, this figure was lower by an average of 30% in I-1 and II-1 main subgroups and did not differ statistically in Group III subgroups (Table 4).

Table 4. Phagocytic activity of neutrophils in umbilical blood of newborns

Study group	Indicators		
	PI,%	PN	PCI
Control	54 (26–70)	3.2 (1.0–4.0)	1.1 (0.8–1.3)
I-1	36*/** (32–42)	3.1 (2.0–3.5)	0.76*/** (0.56–1.0)
I-0	52 (48–56)	2.9 (2.2–3.6)	1.0 (0.9–1.2)
II-1	38*/** (26–44)	2.95 (2.4–3.2)	0.66*/** (0.56–0.88)
II-0	56 (42–64)	2.8 (2.6–4.0)	1.12 (0.80–1.2)
III-1	49 (42–58)	2.9 (2.0–3.3)	0.74*/** (0.62–0.88)
III-0	51 (47–66)	2.6 (2.2–3.6)	0.95 (0.76–1.05)

Note: * the difference is statistically significant compared to the control,
** the difference is statistically significant in subgroups (Kraskel-Wallis test, Mann-Whitney test, $p < 0.05$).

PN in all groups did not go beyond the reference interval, but there was a significantly reduced completeness of phagocytic reactions in all subgroups with implementation of IUI (Table 4). One of the probable factors that increase the risk of fetal IUI, in addition to the level of antigenic load, disruption of the relationship in the system "mother-fetus-newborn", is the lack of both systemic and local immunity of the child [12,13].

Studies have shown a significant decrease in the total number of $CD3^+$T lymphocytes, their subpopulations $CD4^+$T helpers, $CD8^+$T suppressors, as well as $CD25^+$regulatory T-cells in children with complicated early neonatal period, born to women with PNI, compared with newborns with a favorable outcome of IUI.

The numbers of cytotoxic natural killer cells ($CD16^+$ NK), which implement anti-infective protection, in the main groups was higher than in the control, which is apparently due to the effect on the fetus of a large number of antigens. It should be noted that in the groups with the availability of IUI the level of natural killers was significantly higher than in the groups with a favorable outcome of infection.

The percentage of B-lymphocytes in the umbilical blood of newborns with infectious abnormalities was in the range of reference values, but in Group I-0 of newborns the number of cells producing immunoglobulins was significantly higher than in the same group with the IUI availability. According to some authors, in particular I.I. Remizova and co-authors, T.B. Kasokhov and co-authors [12,14], this phenome is associated with the need to increase the production of its own immunoglobulins due to insufficient transplacental transfer of IgG from the mother, because it is known that humoral fetal protection factors are mostly maternal antibodies belonging to the class of IgG antibodies. The content of IgA and IgM in umbilical blood of newborns in the physiological norm is determined in trace concentrations [15].

The increased concentration of IgM in umbilical blood, which was found in various clinical groups, indicates the activation of humoral responses in the newborn in response to an infectious agent and is a marker of IUI. In the groups with IUI implementation, the concentration of IgM and IgG in umbilical blood was lower by 20–30% and 13–17%, respectively, than in similar comparison groups, which is an important factor in

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reducing the anti-infective resistance of newborns. The level of IgA in the clinical groups did not differ statistically, although it exceeded the reference values.

Conclusion. The functional activity of umbilical blood phagocytes in groups with clinical manifestations of IUI was characterized by a significant decrease in the ability of neutrophilic umbilical blood granulocytes to actively absorb and fully digest antigens. Such a defect in the phagocytic protection of the fetus may be one of the probable factors increasing the risk of infection.

Immunological features of umbilical cord blood of newborns born to women with different implementation of intrauterine infection

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Introduction. Perinatal infections (PNIs) have been increasingly referred to as one of the causes of adverse effects of pregnancy recently, accompanied by an inflammatory process, which is based on a highly integrated immune response. Immunopathological reactions cause destabilization of the whole homeostasis and create a favorable basis for the periodic exacerbation of infectious and inflammatory diseases, and the inability of the pregnant woman's body to adequate immune response is the cause of the pathological course of pregnancy. Determination of immunological parameters in the umbilical cord reflects the degree of formation of the immune system of the fetus and thus characterizes the level of anti-infective protection, which depends on the risk of infectious abnormalities. **Objective:** To investigate the immunological features of umbilical cord blood of newborns born to women with different implementation of intrauterine infection (IUI). **Materials and methods.** The study involved examination of 180 pregnant women divided into 3 groups depending on the presence and nature of the detected infection: Group I - viral (CMV, herpes simplex virus types1,2,6), Group II - bacterial (chlamydia, ureaplasma, mycoplasma) and Group III - mixed (viral and bacterial) infection. Each of these groups was divided into 2 subgroups: with subsequent implementation of the infection in newborns (1) and without it (0). This group was the main one in the study. Control group included 50 patients with physiological pregnancy. In clinical trials, samples of umbilical blood obtained during childbirth from women with signs of IUI after the use of different treatment regimens depending on the etiological factor of PNI were studied. **Results and discussion.** Studies have shown a significant decrease in the total number of $CD3^+$T lymphocytes, their subpopulations $CD4^+$T-helpers, $CD8^+$T suppressors, as well as $CD25^+$ regulatory T-cells in newborns with the implementation of IUI. The activity of cytotoxic natural killer cells ($CD16^+$NK), which implement anti-infective protection, was higher in the main groups than in the control, which is apparently due to the effect on the fetus of a large number of antigens. The percentage of B-lymphocytes in the umbilical blood of newborns with infectious abnormalities was in the range of reference values, but in Group I-0 of newborns the number of cells producing immunoglobulins was significantly higher than in the same group with the implementation of IUI, which may be associated with increased production of own immunoglobulins due to insufficient transplacental transfer of IgG from the mother. The increased concentration of IgM in the umbilical blood, which was found in various clinical groups, indicates the activation of humoral responses in the newborn in response to an infectious agent and is a marker of IUI. In the groups with IUI implementation, the concentration of IgM and IgG in the umbilical blood was lower by 20–30% and 13–17%, respectively, than in similar comparison groups, which is an important factor in reducing the anti-infective resistance of newborns. The level of IgA in the clinical groups did not differ statistically, although it exceeded the reference values. **Conclusion.** The functional activity of umbilical blood phagocytes in groups with clinical manifestations of IUI was characterized by a significant decrease in the ability of neutrophilic umbilical blood granulocytes to actively absorb and fully digest antigens. Such a defect in the phagocytic protection of the fetus may be one of the probable factors increasing the risk of infection.

Key words: perinatal infections; implementation of intrauterine infection; umbilical cord blood; immunopathological reactions.

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