Immunological Changes In A Fetus With Hemolytic Disease During Rhesus-Immunization Of The Mother

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Purpose: to assess the effect of Rh immunization on the state of indicators of innate humoral immunity in the fetus, such as immunoglobulins A, M, G, circulating immune complexes of large and small quantities, cytokines IL-1β and IL-8, which play a decisive role in the pathogenesis of the development of a pathological process ...

Materials and Methods: Immunological studies were carried out on 17 umbilical cord blood sera from fetuses with hemolytic disease, which were taken by transabdominal cordocentesis in pregnant women between 24 and 33 weeks of gestation with Rh immunization at the Republican Perinatal Center for 2019 and 2020. All studies were carried out in the laboratory of

immuno-cytokines of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan.

RESULTS: With HDF, the synthesis of immunoglobulins by the fetus increases, primarily due to IgG and IgA, which naturally contributes to an increase in the CEC of both small and large, which are the result of the immunological response of the fetus. The average concentration of IL-1 beta was increased 4.4 times, and the production of IL-8 was significantly suppressed 1.7 times in fetuses with hemolytic disease when compared with the control data.

KEY WORDS: Rh immunization, fetal hemolytic disease (HDF), immunoglobulins A, M, G, small and large circulating immunocomplexes (CIC), cytokines, interleukin-1β, interleukin-8.

1. **RELEVANCE**: The leading place among the immunologically determined pathology during pregnancy is occupied by hemolytic disease of the fetus (HDF) and newborn (HDN). Moreover, in 95% of cases of hemolytic disease develops as a result of incompatibility for the Rh (Rh) factor, and only 5% - for the ABO system [1]. It was found that in subsequent pregnancies, hemolytic disease proceeds more severely than with sensitization detected during the first pregnancy [2].

   In children who have undergone hemolytic disease of newborns, perinatal lesions of the central nervous system, secondary anemia, dysfunction of the hepato-biliary system, allergic diseases often develop in the future; most of them fall into the group of frequently ill children [3]. Currently, thanks to the improvement of methods of prevention, prenatal diagnosis, ante- and postnatal treatment, it was possible to reduce perinatal losses from hemolytic disease, to reduce

the frequency of birth of children with edematous form and deeply premature babies [4].

According to the statistics of the Ministry of Health of the Republic of Uzbekistan, over the past 3 years, the incidence of hemolytic disease in newborns has been growing. There is an upward trend both in percentage terms and in absolute terms. In the structure of perinatal morbidity, if in 2016 HDN occupied 1.6% of the total nosology, in 2017 - 2.1%, then in 2018 this figure reached 2.6%.

This is primarily due to the high birth rate in the republic, the high parity of childbirth among women, the absence of a state program for immunoprophylaxis, the insufficient level of anti-rhesus immunoglobulin administration to women in childbirth by doctors, and the poor development of fetal surgery in our country.

Ultrasound-guided intrauterine intravascular blood transfusion is currently the standard treatment for severe forms of fetal hemolytic disease, which provides effective correction of anemic syndrome in the fetus and allows pregnancy to be prolonged in allo-immunized pregnant women [5].

Despite the studied pathogenetic factors in Rh conflict, immunological changes are still in the focus of attention of immunologists, obstetricians-gynecologists, neonatologists, since Rh sensitization adversely affects the condition of the fetus and newborn [6,7]. Along with the clinical manifestations of Rh-conflict, immunopathological processes are formed, which also adversely affect the immunogenesis of the fetus in utero [8,9].

Many researchers indicate that during physiological pregnancy, the production of pro-inflammatory cytokines is reduced, and the synthesis of anti-inflammatory cytokines is increased, this situation is necessary for the development of the fetus and the formation of its immune system [10,11]. Thus, the available literature well illuminates the results of an immunological study, where presents materials on the study of the population of T- and B-

lymphocytes, as well as complement factors C3 in the umbilical cord blood of the fetus with HD [12,13]. According to their data, a significant suppression of the lymphocyte pool and component C3 of the complement system was revealed, as well as an increase in the content of B-lymphocytes with the CD23 + receptor (receptor for immunoglobulin E) in fetuses, which indicates a response of the cellular link of the adaptive immune system. However, humoral factors, what our research is devoted to remain not fully resolved.

2. **The aim of the study** was to assess humoral immunity factors, such as immunoglobulins A, M, G, circulating immune complexes of large and small values, as well as cytokines IL-1 beta with IL-8, umbilical cord blood of fetuses with hemolytic disease caused by Rh-conflict in pregnant women.
3. **Research materials:**Immunological studies were carried out on 19 umbilical cord blood sera from fetuses with hemolytic disease, which were taken by transabdominal cordocentesis from pregnant women at a period of 24 to 33 weeks with Rh immunization at the Republican Perinatal Center for 2019 and 2020.
4. **IMMUNOLOGICAL RESEARCH METHODS:**

Determination of the level of basic immunoglobulins was carried out by the method of enzyme immunoassay using commercial test systems "Human", Germany. Test systems are based on the sandwich method of enzyme-linked immunosorbent assay using horseradish peroxidase as an indicator enzyme.

Determination of circulating immune complexes of various sizes (CIC) by ELISA on a Stat-Fax analyzer (USA). CEC sizes are determined using different concentrations of PEG. Low concentrations of PEG precipitate large, with a predominance of antigen, high - small CECs with a predominant content of antibodies. Reagents used: 0.1N borate buffer, pH = 8.4; 1.24 g of boric acid is diluted in 100 ml of distilled water; 1.9 g of borax is diluted in 100 ml of distilled water; 4% solution of polyethylene glycol (PEG) m.m. 6000 in distilled water and a 3% solution of polyethylene glycol (PEG) mm

6000 in distilled water. CEC sizes are determined using different concentrations of PEG. Low concentrations of PEG precipitate large, antigen-dominated, high-small CECs with a predominant content of antibodies.

Determination of cytokines reagent kits are a kit, the main reagents of which are MCAbs to the studied cytokines, adsorbed on the surface of the wells of a collapsible polystyrene plate. The kits are intended for the quantitative determination of human cytokines in peripheral blood serum and biological fluids. The measurement of optical density in each well was carried out using an automatic photometer for a microplate at a wavelength of 450 nm by enzyme immunoassay on a Stat-Fax analyzer (USA). For the research were used kits of enzyme-linked immunosorbent assay systems "Human" made in Germany, 2020.

All immunological methods were carried out in the laboratory of immuno-cytokines of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan.

5. **Statistical processing of the results** was carried out using an Excel-2018 program, reflecting the dependence of optical density on concentration for a standard antigen.

6. RESULTS AND DISCUSSION:

The average IgG content was significantly increased in fetal umbilical cord blood serum. Thus, the average concentration of IgG was 12.98 ± 1.87 g / l in a fetus with Rh sensitization, when normally this indicator corresponds to 8.04 ± 0.12 g / l in the fetal umbilical cord blood.

At the same time, the content of IgG in the blood of the fetus is 1.63 times higher than the normative data of the fetus. It is important to note that the maximum IgG value in the group was 26.67 g / l, and the minimum - 3.86 g / l, as you can see, there is a rather large range of values in the group, which is obviously associated with the individual immunoreactive characteristics of the

fetal immune system. Moreover, out of 19 studied samples, 15 (78.9%) had IgG values higher than the normative ones. This fact indicates that the higher the concentration of IgG in the umbilical cord blood, the more active the pathological process caused by Rh immunization. The results of the study are presented in table 1.

Table # 1.

BASIC FETAL SERUM IMMUNOGLOBULINS,

M ± m, g / l

Table 1

The values	**Test values, g / l**	**Standard values, g / l**	**Credibility differences**
Immunoglobulin G	12,98 ± 1,87	8,04 ± 0,12	p<0,05
Immunoglobulin A	0,87 ± 0,46	1,21±0,16	p<0,05
Immunoglobulin M	1,01 ± 0,4	1,08±0,12	p>0,05
CEC 3%, cu	10,41 ± 1,04	6,2±1,12	p<0,05
CEC 4%, cu	20,76 ± 2,02	11,5±1,6	p<0,05

Analysis of the study showed that the average IgM content was insignificant and did not differ significantly when compared with the data of the control group. Thus, the average concentration of IgM was 1.01 ± 0.4 g / l in a fetus with Rh sensitization as compared with the data of the control group, where this indicator was 1.08 ± 0.12 g / l in fetal umbilical cord blood. As you can see, no significant differences were found. Based on the literature data, we can judge that there were practically no women in the group with intrauterine infections, with acute infections during pregnancy. But upon careful analysis, it was revealed that the maximum IgM value in the group was 6.01 g / l, and the minimum was 0.26 g / l, and there was a high standard value in only two

samples. The data obtained confirm the scientific potential of research, and dictate the need for further more in-depth research in this direction.

Further, the serum concentration of IgA was studied where it was found that its average content was also significantly increased in the serum of the umbilical cord blood of the fetus. Thus, the average concentration of IgA was 0.87 ± 0.46 g / l in a fetus with Rh sensitization compared with the data of the control group, where this indicator was 1.37 ± 0.16 g / l in the umbilical cord blood of a fetus of healthy women. At the same time, the average IgA content in the fetal blood was reduced by 1.57 times when compared with the data of the control group of fetuses. It is important to note that the maximum IgA value in the group was 4.96 g / l, and the minimum - 0.013 g / l; a sufficiently large range of values within the group was revealed, which apparently indicates the individual characteristics of the fetal immune system, which develops under these conditions. Of the 19 studied samples, only 3 (15.8%) had IgA values higher than the normative ones, they were mainly suppressed, due to which the average IgA content in this group was significantly reduced. Thus, the reduced IgA values indicate the presence of a deficiency of one of the links of the humoral factors of fetal immunity, which is obviously caused by an immunological conflict, the solution of which will help to improve the humoral immunity of the fetus.

Large and small CECs were studied. Each of them has important diagnostic and prognostic value. The results of the study are also presented in table 1.

It was shown that in the experimental group the average content of the CEC of large values was 10.41 ± 1.04 a.u., while in the control group it was 6.2 ± 1.12 a.u., which was significantly increased in the experimental group with comparison with the data of the control group. At the same time, the content of large CEC values was increased in the fetal blood by 1.7 times when compared with the data of the control group. Also, it should be noted that the maximum

value of the CEC of large values in the group was 20 conventional units, and the minimum - 4 conventional units, and at the same time, 12 (63.1%) had increased values.

Further, small-sized CECs were studied. It was shown that in the experimental group the average content of the CEC of small values was 20.76 ± 2.02 a.u., while in the control group it was 11.5 ± 1.6 a.u., which was also significantly increased in the experimental group. when compared with the data of the control group. At the same time, the content of CEC of small values is increased in the blood of the fetus by 1.8 times in comparison with the data of the control group. As you can see, the results turned out to be quite diagnostic, which may later serve to create algorithms and protocols for immunodiagnostics of severity and prognosis of immunological Rh-conflict. Also, it should be noted that the maximum value of the CEC of small values in the group was 34 conventional units, and the minimum - 10 conventional units, and at the same time, in 16 samples (84.2%) increased values were revealed.

Analysis of serum concentrations of interleukins IL-1 beta showed that the average concentration in the fetus was 14.65 ± 2.91 pg / ml, while in the control group it was 3.35 ± 0.12 pg / ml, the differences were significant. It can be seen that the average concentration of IL-1 beta was increased 4.4 times. If we analyze the maximum values that were in the group, we can note that the maximum value was 44.15 pg / ml, and the minimum was 3.29 pg / ml, which indicates the presence in the group of samples with increased values, which naturally influenced the average concentration. This situation is absolutely correct from the side of statistics, and once again confirms that the immunoreactivity of both the fetus and the adult is absolutely individual and heterogeneous. Elevated IL-1 beta values were found in 17 (89.5%) samples from 19, which indicates the presence of an immune response formed in response to the antigen. The results obtained are presented in table 2.

7. ESSENTIAL FRUIT SERUM CYTOKINES,

M ± m, pg / l

Table No 2

The values	**The investigated values of the fetus, pg / l**	**Standard values of the fetus, pg / l**	**Credibility differences**
Interleukin-1 Betta	14,65 ± 2,91	3,35± 0,12	p<0,05
Interleukin-8	37,30 ± 7,03	62,4 ± 2,12	p<0,05

In turn, the analysis of serum concentrations of interleukins IL-8 showed that the average concentration in the fetus was 37.30 ± 5.03 pg / ml, when compared with the data of the control group - 62.4 ± 2.12 pg / ml, as can be seen, the difference in values are reliable enough. And at the same time, we revealed a significant suppression of the production of IL-8, which is a powerful chemokine cytokine that promotes the formation of adaptive mechanisms of the fetus after birth. The suppression of IL-8 when compared with the control data was 1.7 times. If we analyze the maximum values that were in the group, we can note that the maximum value was 129.37 pg / ml, and the minimum was 7.33 pg / ml. Moreover, suppression of IL-8 was also detected in 17 (89.5%) of 17 samples, which indicates immunosuppression.

8. CONCLUSIONS:

1. In Rh-conflict, the synthesis of immunoglobulins by the fetus increases, primarily due to IgG and IgA, which naturally contributes to an increase in the CEC, which are the result of the immunological response of the fetus. An increase in IgG and prolonged circulation of antigen promoted the formation of immune complexes, which damage tissues.

2. High CEC indicators of both large and small values are evidence that the fetus has an excess of antigens that have penetrated into their body in the prenatal period, to which an immunological reaction is played, as mentioned above, the cytotoxic type of immune response. Moreover, small CECs have a pronounced pathological potential.

3. Increased values of IL-1 beta in fetuses with hemolytic disease indicate the presence of a pronounced immune response in the fetus associated with immune reactivity to the mother's antigen, which contributes to the activation of innate immunity components, thereby activating the pro-inflammatory cytokine cascade.

4. Decreased IL-8 values are a marker of deficiency of chemokine activation of innate immunity cells. The revealed two-fold suppression of IL-8 production indicates the suppression of maturation and activation of neutrophils, apoptosis and migration of neutrophilic leukocytes, as well as hypoxia in the fetus.

9. REFERENCES

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