HUMORAL IMMUNITY OF THE FETUS IN PREGNANT WOMEN WHO HAVE UNDERGONE COVID-19 IN VARIOUS FORMS.

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ABSTRACT
The purpose of this research is to provide the findings of a study of immunoglobulins A, M, G, and fetuses in pregnant women who had COVID-19 with both a moderate and severe course of the disease.
In 2022, the Republican Perinatal Center conducted immunological research on 70 fetal cord blood sera obtained by transabdominal cordocentesis in pregnant mothers with COVID-19. Pregnant women are categorized into three categories. Group I (control) consists of pregnant women with physiological pregnancy (n=10), whereas Group II consists of pregnant women who had COVID-19 in the second trimester of their pregnancy (n=30). Group III consists of pregnant women who had COVID-19 in the third trimester of their pregnancy (n=30). The Institute of Immunology was the site of all immunological research.
The level of IgG in the blood of fetuses against the background of severe COVID-19 of the mother was significantly increased by 2.3 times and 2 times compared to the control group in the II and III trimesters. The content of IgM in fetuses against the background of a severe course of COVID-19 in mothers was significantly increased compared to the control data by 2 times both in the II and III trimesters. With an average course of the disease, the indicators of all immunoglobulins A, M, G, in fetuses were not significantly changed compared to the normative data in both the II and III trimesters.
In pregnant women who underwent COVID-19 in severe form both in the second trimester and in the third trimester of gestation, the synthesis of immunoglobulins by the fetus increases due to IgG and IgM, which are the result of the immunological response of the fetus.
KEYWORDS: COVID-19, immunoglobulins A, M, G, II, III-trimester of pregnancy.

INTRODUCTION

Most clinicians have confirmed that a pregnant woman with coronavirus pneumonia can predispose patients to a more rapid deterioration of the clinical course and can lead to a

maximum risk of harm to both the mother and fetus, which is often associated with changes in hormone levels and a decrease in lung volume due to a pregnant uterus and an immunodeficiency condition [1,2].

A number of authors, having conducted a meta-analysis of maternal and perinatal outcomes in COVID-19 in pregnant women, conclude that the risk of vertical transmission is low and may not depend on the severity of the mother's disease [3].

Severe coronavirus infection is an indication in pregnant women for delivery if it is necessary to improve the oxygenation of the mother. The transmission of coronavirus from a pregnant woman to a fetus has also not been proven. The virus is absent in breast milk and therefore experts recommend breastfeeding for the acquisition of protective antibodies in newborns [4].

Pregnant women with COVID-19, compared with pregnant women without COVID-19, are more likely to give birth prematurely and have an increased risk of maternal mortality and hospitalization in the intensive care unit. Their children are more likely to be placed in the neonatal unit [5].

Studies have shown that pregnant women with a positive result for COVID-19 had a higher number of leukocytes, neutrophils, monocytes, as well as premature births were more common in COVID-19-positive pregnant women accompanied by a significantly lower birth weight (2894.37 (± 67.50) g compared with 3194.16 (± 50.61) g, p = 0.02) in COVID-19-negative pregnant women [6].

It is known that immunoglobulins are the most important components of humoral specific immunity, representing globular plasma proteins secreted by plasma cells of the immune system and designed to neutralize all antigenic structures [7,8]. Immunoglobulin M is the first immunoglobulin that begins to be synthesized in a human fetus (approximately at 18-20 weeks). Immunoglobulins M can interact with component C1 of the complement system and activate the classical pathway of the complement system, resulting in opsonization of antigens and cytolysis [9, 10]. Due to the large size of immunoglobulin M, they do not pass through the placenta, but can be synthesized by placental cells [11].

Thus, the literature data listed above and our knowledge in this field necessitate a comprehensive study of the role of congenital humoral immunity of the fetus in the womb of a mother who has suffered an acute respiratory infection, in particular COVID-19.

The aim of the study was to evaluate the main fetal immunoglobulins A, M and G in the umbilical cord blood of fetuses in pregnant women who underwent COVID-19, depending on the severity of the mother's disease.

MATERIALS AND METHODS

Immunological studies were conducted on 70 fetal cord blood sera taken by transabdominal cordocentesis in pregnant women who underwent COVID-19 in the second trimester of gestation at the Republican Perinatal Center in 2022. All pregnant women are divided into 3 groups. Group I (control) – pregnant women with physiological pregnancy and without a burdened obstetric and somatic history (n=10), group II - pregnant women who underwent COVID-19 in the second trimester of their gestation (n=30). Group III- pregnant women who underwent

COVID-19 in the third trimester of their gestation (n=30). Fetal blood sampling was performed using transabdominal cordocentesis in the period from 18 to 34 weeks of gestation.

In order to compare the obtained results of immunoglobulins with the values of the fetus, we used material that was collected from pregnant women in the second trimester of pregnancy, who were directed to this manipulation due to various reasons. All the collected material was frozen and stored. To set the standard values, we used pregnant women who did not have perinatal pathology. In order to compare the results of the study in the 3rd trimester of pregnancy, we used normative data, because they are quite well represented in the international literature.

Immunological research methods:

All studies were conducted in the laboratory of immunocytokines of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan. Determination of the level of the main immunoglobulins was carried out by the method of enzyme immunoassay using commercial test systems "Human", Germany. The test systems are based on the sandwich method of solid-phase enzyme immunoassay using horseradish peroxidase as an indicator enzyme.

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

RESULTS AND DISCUSSION

The contents of the main serum immunoglobulins in the fetus in different trimesters of development and depending on the severity of the infectious process were studied. The data are presented in tables №1**.**

Table-№1.

Serum immunoglobulins of the fetus of a mother who underwent covid-19 in the second trimester of pregnancy

Values	**The average current of COVID-19**	**M±m, g/l Heavy current**	**COVID-19**
Immunoglobulin G	4,85 ± 1,53	7,26 ± 1,32*^	3,17 ± 0,23
Immunoglobulin A	0,88 ± 0,16	1,21 ± 0,11*^	0,82 ± 0,14
Immunoglobulin M	1,74 ± 0,14*	1,92 ± 0,33*	0,95 ± 0,42

Note: * - the reliability of differences with the control group, ^ - between the studied groups ($p < 0.05$).

Thus, the analysis of the content of the main serum immunoglobulins of the fetus in the second trimester of pregnancy, depending on the severity of the course of COVID-19 transferred by the mother, presented in Table No. 1 showed that the average content of **immunoglobulin G in the fetus** in the group with severe COVID-19 transferred in mothers was significantly increased compared with the control data with with medium-heavy current. In the control group, this indicator corresponded to 3.17 ± 0.23 g / l, and in the fetus it was increased by 1.5 times

compared with the data with a moderate course, and with the control data – by 2.3 times. The average concentration of immunoglobulin G in this group was 7.26±1.32g/l.

As for the index of changes in adaptive humoral mechanisms of the fetus at this time, it should be noted that during this period the fetal immune system produces a very low content of immunoglobulins, including immunoglobulin G. Therefore, our index will be significantly reduced. So, if normally we can imagine that this will be IgG/IgA – 3.8, and in a mother's fetus with a severe course – 6, with a moderate course - 5. IgG/IgM was normally 3.3, and in a fetus with a severe course – 3.8, with a moderate course – 2.8.

Thus, the analysis of the content of serum **immunoglobulin A of the fetus** in the second trimester of pregnancy, depending on the severity of the course of COVID-19 suffered by the mother, presented in Table No. 1, showed that its average content in the group with severe COVID-19 in mothers was significantly increased compared with control data and with data with moderate the current. In the control group, this indicator corresponds to 0.82 ± 0.14 g/l. In a fetus with a severe course of COVID-19 in mothers, compared with the data with a moderate course, it increased by 1.4 times, and with control data – by 1.5 times. This indicates an increase in the humoral nonspecific factor responsible for the infectious process against the background of a pregnant woman with COVID-19 in the second trimester. The average concentration of immunoglobulin A in the severe group was 1.21 ± 0.11 g/l. Such an increase in immunoglobulin A in the fetus is due to the direct production of its own immunoglobulin A, and is a reflection of the infection suffered by the mother.

Further, the analysis of the content of the main serum immunoglobulins of the fetus in the second trimester of pregnancy, depending on the severity of the course of COVID-19 suffered by the mother, presented in Table No. 1 showed that the average content of immunoglobulin M in the fetus in the group with severe COVID-19 in mothers was also significantly increased compared to the control data, but not with a value with a moderate current. In the control group, this indicator corresponds to 0.95 ± 0.42g/l, and in a fetus with a severe course of COVID-19 in mothers, it was increased by 1.1 times compared to fetuses with a moderate course and was not significantly distinguishable. With the control data, the content of immunoglobulin M in the fetus was increased by 2 times. This indicates that this immunoglobulin M is produced by plasma cells of the fetal immunity itself. Thus, the average concentration of immunoglobulin M in the group with severe course was 1.92±0.33g/l. Obviously, such an increase in immunoglobulin M in the fetus is a reflection of the transferred infection, it can be both the mother and the fetus itself.

Studies have also been conducted on the content of the main serum immunoglobulins in the fetus in the third trimester of pregnancy, depending on the severity of the infectious process. The data is presented in Table №. 2.

Table №2.

Serum immunoglobulins of the fetus of a mother who underwent covid-19 in the third trimester of pregnancy

Values	**The average current of COVID-19**	**M±m, g/l Heavy current**	**COVID-19**
Immunoglobulin G	9,74 ± 1,23	11,68 ± 1,21*	8,57 ± 1,32
Immunoglobulin A	1,34 ± 0,23	1,52 ± 0,12	1,30 ± 0,33
Immunoglobulin M	1,68 ± 0,23*	1,79 ± 0,15*	1,45 ± 0,24

Note: * - the reliability of differences with the control group ($p < 0.05$).

Thus, the analysis of the main serum immunoglobulins of the fetus in the third trimester of pregnancy, depending on the severity of the course of COVID-19 suffered by the mother, presented in Table No. 2, showed that the content of immunoglobulin G in the fetus in the group with severe COVID-19 in mothers was significantly increased compared with the normative data. If the average value of the normative data corresponds to 8.57 ± 1.32 g / l, then in a fetus with a severe course of COVID-19 in mothers, this indicator is increased by 1.4 times, and with data with a moderate course by 1.2 times, but not reliably. The average concentration of immunoglobulin G in the severe group was 11.68±1.21 g/l. Obviously, such an increase in immunoglobulin G in the fetus is a reflection of the infection suffered by the mother and most likely the development of immunity by the fetus itself, the formation of maternal specific immunity in the mother and the circulation of immunoglobulins G in the fetal bloodstream, since immunoglobulin G penetrates the placental barrier.

The content of fetal immunoglobulin A in the group with severe COVID-19 in mothers was increased in comparison with the normative values and with the data with a moderate course, but not significantly. The normative values correspond to 1.30 ± 0.33 g / l, and in a fetus with a severe course of COVID-19 in mothers, this indicator is increased by 1.2 times, and compared with data with a moderate course by 1.14 times. The average concentration of immunoglobulin A in this severe group was 1.52±0.12 g/l.

The average content of immunoglobulin M in the fetus in the group with severe COVID-19 in mothers was also significantly increased compared to the normative data, but not with the value with a moderate course, although there is a significant difference between the values of the fetus and the normative data. The normative values correspond to 1.45 ± 0.24 g / l, and in a fetus with a severe course of COVID-19 in mothers, it is unreliably increased compared to moderate data, and with normative data it is increased by 1.3 times. This indicates a slight increase in the humoral nonspecific factor responsible for the acute infectious process. Thus, the index of immunoglobulin M in this group was 1.79±0.15g/l. Obviously, such an increase in immunoglobulin M in the fetus is a reflection of the transferred infection, it can be both the mother and the fetus itself.

Thus, the IgG/IgA ratio was normal – 6.6, and in the fetus of a mother with a severe course – 7.6, with a moderate course – 7.3. IgG/IgM was normal – 5.9, and in a fetus with a severe course – 6.5, with a moderate course – 5.8. This picture indicates an increase in IgG

immunoglobulin in fetal blood, most likely due to maternal immunoglobulins G, and insignificant production of own immunoglobulins A and M of the fetus.

CONCLUSION

- the content of immunoglobulin G in the fetus against the background of severe COVID-19 in mothers in the second trimester was significantly increased compared to the control data by 1.5 times, and with the data with a moderate course by 2.3 times.
- immunoglobulin A in the fetus against the background of a severe course of COVID-19 in mothers in the second trimester was significantly increased 1.5 times compared with the control data, and 1.4 times with a moderate course.
- the analysis of the content of immunoglobulin M in the fetus against the background of severe COVID-19 in mothers in the second trimester was significantly increased by 2 times compared with the control data.
- the content of immunoglobulin G in the fetus against the background of severe COVID-19 in mothers in the third trimester was significantly increased by 1.4 times compared with the normative data.
- the average concentration of immunoglobulin M in the fetus against the background of severe COVID-19 in mothers in the third trimester was significantly increased by 1.3 times compared with the normative data.

REFERENCES:

1. Dong L, Tian J, He S, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. JAMA 2020

2. Kara B. Could Maternal COVID-19 Disease be a Risk Factor for Neurodevelopmental Disorders in the Child? Turk Arch Pediatr. 2021 Nov;56(6):542-544.

3. Bellos I, Pandita A, Panza R. Maternal and perinatal outcomes in pregnant women infected by SARS-CoV-2: A meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2021 Jan; 256:194-204.

4. Rajewska A, Mikołajek-Bedner W, LebdowiczKnul J, Sokołowska M, Kwiatkowski S, Torbé A. COVID-19 and pregnancy - where are we now? A review. J Perinat Med. 2020 Jun 25;48(5):428-434

5. Allotey, J., Stallings, E., Bonet, M., et al. (2020) Clinical Manifestations, Risk Factors, and Maternal and Perinatal Outcomes of Coronavirus Disease 2019 in Pregnancy: Living Systematic Review and Meta-Analysis. BMJ, 370, m3320. https://doi.org/10.1136/bmj.m3320

6. Marwah M. it all. SARS-2 COVID-19-induced immunity response, a new prognostic marker for the pregnant population correlates inversely with neonatal Apgar score. Infection. 2022 Mar 5:1–9.

7. Bakhodirova Sh. F., Ikhtiyarova G. A., Aslonova M. J., Davlatov S. S. (2020). Features of perinatal outcomes in women after supporting reproductive technologies. European Journal of Molecular & Clinical Medicine, 7(2), 6350-6356.

8. Ikhtiyarova, G. A., Dustova, N. K., Khasanova M. A., Suleymanova G. S., & Davlatov, S. S. (2021). Pathomorphological changes of the placenta in pregnant women infected with

coronavirus COVID-19. International Journal of Pharmaceutical Research, 13(1), 1935-1942. doi: 10.31838/ijpr/2021.13.01.283

9. Cao W.C., Liu W., Zhang P.H., Zhang F., Richardus J.H. Disappearance of antibodies to SARS-associated coronavirus after recovery. N. Engl. J. Med., 2007, Vol. 357, no. 11, pp. 1162-1163.

10. Azkur A.K., Akdis M., Azkur D., Sokolowska M., van de Veen W., Brüggen M.-C., O'Mahony L., Gao Y., Nadeau K., Akdis C.A. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy, 2020, Vol. 75, no. 7, pp. 1564-1581.

11. Kudratova, D. Sh, Ikhtiyarova, G.A., & Davlatov, S.S. (2021). Medical and social problems of the development of congenital malformations during a pandemic. International Journal of Pharmaceutical Research, 13(1), 756-760. doi: 10.31838/ijpr/2021.13.01.130

12. Carrillo J., Izquierdo-Useros N., Avila-Nieto C., Pradenas E., Clotet B., Blanco J. Humoral immune responses and neutralizing antibodies against SARS-CoV-2; implications in pathogenesis and protective immunity. Biochem. Biophys. Res. Commun., 2021, Vol. 538, pp. 187-191.

13. Baumgarth N., Nikolich-Zugich J., Lee F.E.-H., Bhattacharya D. Antibody responses to SARS-CoV-2: let's stick to known knowns. J. Immunol., 2020, Vol. 205, no. 9, pp. 2342-2350.