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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

DIAGNOSIS OF IMMUNOLOGICAL CONFLICT DURING PREGNANCY AND THE EFFECTIVENESS OF INTRAUTERINE BLOOD TRANSFUSION IN RHESUS ISOIMMUNIZATION

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Field of science: Medicine

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GENERAL CHARACTERISTICS OF THE WORK

Relevance of the Topic In modern medicine, the diagnosis and proper treatment of hemolytic disease of the fetus (HDF) remain pressing issues. Modern scientific and medical research indicates that the incidence of HDF ranges from 3% to 6%, contributing significantly to perinatal mortality among newborns[1]. It is well known that the most complex immunological interactions between the mother and fetus develop during the early weeks of pregnancy[2].

The pathogenesis of Rh incompatibility is primarily driven by the transfer of fetal erythrocytes across the placental barrier into the maternal bloodstream. In Rh-negative mothers, the immune system produces antibodies against Rh-positive fetal erythrocytes, leading to an antigen-antibody reaction-the fundamental mechanism behind hemolytic disease of the fetus.

Key factors that contribute to Rhesus immunization, include: Previous transfusion of Rh-positive blood; Spontaneous abortion; Induced or medical abortion; Ectopic pregnancy.

The titer of Rh antibodies ranging from 1:4 to 1:16 typically indicates the absence of hemolytic disease in newborns, or at most, a mild form. In more severe cases, when the Rh antibody titer ranges from 1:16 to 1:4098, fetuses are more likely to be born with moderate to severe forms of hemolytic disease. In such cases, the likelihood of prenatal morbidity in newborns increases significantly, and the incidence of death is generally higher[3].

In cases of moderate to severe hemolytic disease of the fetus

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1 Лялькова, И.А., Галиаскарова, А.А., Байтанатова, Г.Р. Прогностическое значение допплерометрии мозгового кровотока в диагностике гемолитической болезни плода // –Москва: Журнал Актуальные вопросы акушерства гинекологии и перинатологии,– 2013. – p. 88-90.

2 Transfusion of least-incompatible blood with intravenous immunoglobulin plus steroids cover in two patients with rare antibody / N.Win, M.Needs, N.Thornton [et al.] // Transfusion, – 2018. №7(58), – p. 1626-1630.

3 Абдрахманова, Л.Р., Токтарова, О.А., Ситарская, М.В. Анализ результатов доплерометрического исследования кровотока в средней мозговой артерии у плода при резус-иммунизации // – Казань: Журнал «Практическая медицина», –2015. № (4) 89, –p. 7-9.

and newborn, a monotonous increase in antibody titer is typically noted. In more severe forms, a sudden (jump-like) increase in antibody titer is observed[4].

Hemolytic disease in the fetus is primarily manifested as chronic hypoxia. The degree of hypoxia is directly related to the severity of the hemolytic disease[5].

In these cases, it is recommended to monitor the antibody levels in the mother's blood monthly until the 28th week of pregnancy, and twice a month after the 28th week[6].

Symptoms of isosensitization in the mother are reflected in the fetus through anemia, hypoxia, and hyperbilirubinemia. It has been shown that testing for antibodies in the mother's blood may not always provide high diagnostic sensitivity. Even if antibodies are present, if the fetus is Rh-negative in the next pregnancy, hemolytic disease of the fetus (HDF) may not develop[7].

Scientific studies have determined that at low antibody titers (ranging from 1:2 to 1:16), newborns are either healthy or have mild hemolytic disease. At high antibody titers (ranging from 1:32 to 1:2048 or 1:4096), severe forms of HDF are typically observed. However, it is also noted that severe forms of HDF can occur even at low titers of antibodies, which is influenced by the barrier function of the placenta.

In modern perinatal diagnostics, the peak systolic velocity (PSV) of blood flow in the middle cerebral artery (MCA) of the fetus (measured by Doppler ultrasonography) is an important diagnostic

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4 Гемолитическая болезнь плода у беременных с резус- сенсибилизацией. Диагностика, лечение, профилактика / Г.М. Савельева, М.А. Курцер, О.Б. Панина [и др.]: [E-resource] /Министерство Здравоохранения Российской Федерации, –18 мая, 2017. URL: https://docs.cntd.ru/document/ 902328227

5 Hemolytic disease of the fetus and newborn due to Rh(D) incompability: a preventive disease that still produces significant morbidity and mortality in children / V.Pegoraro, D.Urbinati, G.H.A. Visser [et al.]: [Electron resource]/ PLoS ne.,2020.№7(15), URL:https://pubmed.ncbi.nlm.nih.gov/32687543/

6 Жабборов У.У., Расуль-Заде Ю.Г. Новый взгляд на проблему резус-иммунизации в Республике Узбекистан // Ж. Медицинские новости, – 2020, №4, – p. 83-86.

7 Li, S. Fang, Q. Hyporegenerative anemia in anti-M-associated hemolytic disease of the fetus // Transfusion, – 2021. Vol. 61, №6, –pp. 1908-1915.

indicator. These measurements have been found to correlate strongly with the hemoglobin concentration in the fetus. An increase in MCA-PSV indicates heightened hyperdynamic blood circulation in the fetus. An increase in this indicator above 1.5 MoM suggests severe fetal anemia.

In severe cases of fetal hemolytic disease, intrauterine blood transfusions are administered to the fetus. Indications for intrauterine blood transfusions to the fetus include ultrasound findings and determination of Hb and Hct levels via cordocentesis. According to literature data, intrauterine blood transfusions to the fetus are performed once in 50% of cases, twice in 15% of cases, and three or more times in 35% of cases.

According to the authors, intrauterine hemotransfusion of the fetus via transplacental umbilical cord access is uncomplicated in 93% of cases, and complicated in 7% of cases.

7%, transamniotic umbilical cord placental access without complications

75%, complicated – 25%, free loop access without complications – 77%, complicated – 23%, intrahepatic access without complications – 92%, complicated – 8%.

It should be noted that there are no scientific studies in Azerbaijan on the isosensitization of pregnant women to the Rh factor, and, to date, no research has been conducted on intrauterine hemotransfusion of the fetus. The purpose of the current study was determined taking into account the relevance of the problem.

The object and subject of the study. The study was conducted in Baku city. Between 2015 and 2021, 77 Rh-negative pregnant women were examined at the Departments of Obstetrics and Gynecology I and II, as well as the Teaching Surgery Clinic of Azerbaijan Medical University. The mean age of pregnant women was 30.53±0.61 years and ranged from 18-43.

The aim of the study is to evaluate the effectiveness of modern diagnostic methods in pregnant women with Rh isoimmunization and the efficacy of intrauterine blood transfusion in severe forms of fetal hemolytic disease.

Objectives of the study:

Study of pregnancy progression, birth outcomes, and the health of the fetus and newborn in women without rh negative sensitization.

Determining the risk factors and incidence of Rhesus isosensitization, study of pregnancy characteristics in women with rh sensitization.

Evaluation of the diagnostic effectiveness of non-invasive methods (ultrasound and doppler imaging) in diagnosing fetal hemolytic disease in women with rh sensitization.

Study of the indications, techniques, treatment effectiveness, and impact on the fetus and mother of intrauterine blood transfusion in severe forms of fetal hemolytic disease.

Laboratory tests after intrauterine hemotransfusion of the fetus and evaluation of functional indicators: study of early neonatal characteristics in newborns born after intrauterine blood transfusion and its impact on perinatal outcomes.

Main statements for the defence of the thesis:

Risk factors for Rh sensitization include failure to administer anti-D immunoprophylaxis after previous births, miscarriages (including premature, spontaneous, and induced abortions), a history of antenatal fetal death (AFD), vaginal bleeding during pregnancy, and incorrect determination of the mother's Rh status.

Anti-D immunoprophylaxis in Rh-negative women is an effective preventive measure against fetal hemolytic disease, positively influencing the course of future pregnancies, as well as the health of the mother, fetus, and newborn.

In women with Rh sensitization, 100% fetuses develop hemolytic disease during the third trimester of pregnancy. Using non-invasive methods, hemolytic disease of the fetus is detected at an average of 25.1±1.3 weeks of gestation in the second trimester, and at 30.0±1.2 weeks in the third trimester.

Echographic criteria for fetal hemolytic disease include pathological increase in placental thickness, enlargement of the fetal spleen and liver, increased ratio of fetal heart circumference to thoracic circumference, fetal edema, ascites, double contour of the fetal scalp, umbilical cord edema, and venous dilatation. Doppler examination shows a maximum systolic blood velocity in the middle

cerebral artery of 71.63 ± 3.59 cm/s in the second trimester and 80.2 ± 2.15 cm/s in the third trimester (>1.5 MoM), indicating fetal hemolytic disease.

The presence of criteria for fetal hemolytic disease through non-invasive methods is an indication for amniocentesis and cordocentesis. Conducting genetic and microbiological examinations using amniocentesis is essential.

A decrease in fetal hemoglobin, hematocrit, and platelet levels detected by cordocentesis indicates the need for intrauterine blood transfusion. Intrauterine blood transfusion in patients with fetal hemolytic anemia is highly effective and significantly reduces perinatal morbidity and mortality.

Scientific novelty of the study: The conducted research examined the course of pregnancy, the effects of childbirth on the mother and newborn in women without Rh sensitization and in pregnancies with anti-D immunoprophylaxis. The risk factors and frequency of Rhesus isosensitization have been studied.

Echographic and Doppler criteria for fetal hemolytic disease during pregnancy dynamics have been established using non-invasive methods.

Invasive prenatal diagnostic methods were used to assess the severity of fetal hemolytic disease, along with genetic and microbiological examinations of amniotic fluid. For the first time in the Republic of Azerbaijan, the technique, methods of implementation, and effectiveness of intrauterine blood transfusion in severe forms of fetal hemolytic disease have been studied. As a result of the study, perinatal mortality rates decreased from 100% to 23.1%.

Theoretical and practical significance of the study: The findings emphasize the importance of conducting anti-D immunoprophylaxis in Rh-negative women, regardless of the course of pregnancy. In Rh-negative women, anti-D immunoprophylaxis is mandatory at 28.7 ± 0.9 weeks of pregnancy.

It was determined that Rh-negative women had repeated pregnancies with factors leading to Rh sensitization, including a history of recurrent or incomplete pregnancies, lack of anti-D immunoprophylaxis after delivery, vaginal bleeding for various reasons during pregnancy, a history of induced and

spontaneous abortions, and incorrect determination of the mother's Rh status.

If the newborn is Rh-positive during early pregnancy, it is essential to administer anti-D immunoglobulin prophylaxis within 72 hours. Ultrasound and Doppler imaging during pregnancy in Rh-negative women enable the non-invasive diagnosis of fetal hemolytic disease. The study examined the indications, techniques, and effectiveness of intrauterine blood transfusion performed via amniocentesis and cordocentesis. Based on the findings, intrauterine transfusion is recognized as a highly effective treatment for fetal hemolytic disease, significantly influencing on perinatal outcomes.

Approbation and Application of the Research:

The list of scientific conferences where the research results were presented in are below:

1. Karabakh III international congress of applied sciences "Year of Shusha-2022", June 7-10, Karabakh, Azerbaijan.
2. Современная медицина: новые подходы и актуальные исследования, Москва, 23.08.2022
3. "Pediatriyanın aktual problemləri" XII beynəlxalq Elmi-Praktiki Konqres Tezis Toplusu, 11-13 oktyabr, 2022, Bakı.
4. Ümumilli Lider Heydər Əliyevin anadan olmasının 100 illiyinə həsr olunmuş "Təbabətin Aktual Problemləri" Beynəlxalq elmi-praktiki konqres, 3-6 may, Bakı.
5. Azərbaycan xalqının Ümummilli lideri HEYDƏR ƏLİYEVİN anadan olmasının 100 illiyinə həsr olunan rezident və magistrantların 11-ci ELMİ TƏCRÜBİ KONFRANSI, ATUREK-11, konfrans materialları, Bakı – 2023.
6. TİBB ELMLƏRİ DOKTORU, ƏMƏKDAR ELM XADİMİ, ŞÖHRƏT ORDENLİ PROFESSOR BƏYBALA XUDKAR OĞLU ABBASOVUN 100 İLLİK YUBİLEYİNƏ HƏSR OLUNMUŞ ELMİ-PRAKTİK KONFRANSIN MATERİALLARI, Bakı-2023.

The research was presented at a meeting of the I Department of Obstetrics and Gynecology at Azerbaijan Medical University (AMU)

on February 4, 2022 (Protocol №3), between departments on July 5, 2023 (Protocol №3) and ED 2.06 scientific seminar of the dissertation council on June 14, 2024 (Protocol №12) where the scientific findings were discussed.

The author has published seven articles on the topic in reputable scientific journals, including three in international journals. Additionally, the research was featured in presentations at both international and national scientific conferences, resulting in the publication of 7 abstracts and reports in conferences.

Institution where the dissertation was conducted: The dissertation was carried out at the First and Second Departments of Obstetrics and Gynecology within the Teaching and Surgical Clinic of Azerbaijan Medical University.

Structure and Scope of the Dissertation: The dissertation is composed of an introduction, three main chapters, and a conclusion. It includes 36 tables, 6 graphs, and 6 images. The total scope of the dissertation is 175,651 characters, distributed as follows: introduction – 15,013 characters; Chapter I – 52,712; Chapter II – 12,687; Chapter III – 34,998; and conclusion – 60,273 characters.

MATERIALS AND METHODS OF THE STUDY

77 Rhesus (Rh) negative pregnant women were examined. The participants ranged in age from 18 to 43 years, with an average age of 30.53 ± 0.61 years.

Throughout the course of pregnancy, Rh-negative pregnant women underwent ultrasound assessments of the fetus, amniotic fluid, and placenta, as well as Doppler ultrasound examinations of the umbilical artery. Additionally, Doppler measurements were used to determine the peak systolic velocity in the middle cerebral artery. An indirect Coombs test was performed to determine antibody titer in pregnant women. Based on the results of ultrasound and Doppler studies during the pregnancy, invasive procedures such as amniocentesis and cordocentesis were conducted in cases of suspectted severe fetal anemia. Furthermore, microbiological and genetic analyses were performed on the amniotic fluid obtained during amniocentesis.

Ultrasound Examination

Starting from 7–8 weeks of pregnancy, ultrasound evaluations were performed in Rh-negative women. These assessments included monitoring embryonic and fetal development, fetometric parameters such as biparietal diameter, abdominal circumference, femur length, as well as measurements of amniotic fluid volume and placental thickness, up to 38 weeks of pregnancy.

To assess maternal-placental and fetal-placental circulation, fetal umbilical blood flow and the peak systolic velocity in the middle cerebral artery were measured.

Invasive Diagnostic Methods

Amniocentesis is an invasive procedure in which a 20G spinal needle is inserted through the anterior abdominal wall into the uterine cavity under ultrasound guidance to collect a sample of amniotic fluid for analysis. This procedure is typically performed from the 15th week of gestation. The collected fluid was subjected to microbiological and genetic testing.

Microbiological Analysis of Amniotic Fluid

Infections such as cytomegalovirus (CMV), parvovirus B19, and toxoplasmosis were detected in the amniotic fluid using polymerase chain reaction (PCR) testing.

Genetic Analysis of Amniotic Fluid

Analysis of the amniotic fluid enabled the determination of the fetal karyotype, and no chromosomal abnormalities were identified.

Cordocentesis

Cordocentesis is an invasive procedure in which a 22G spinal needle is inserted into the fetal umbilical vein under ultrasound guidance to collect 2 ml of fetal blood. It is generally performed from the 18th week of gestation. Cordocentesis was carried out from the 20th week onward to diagnose fetal hemolytic disease. The obtained blood samples were analyzed to determine fetal hemoglobin (Hb), hematocrit (Hct), and platelet count (Plt). If necessary, additional samples were taken following blood transfusion.

RESULTS OF THE PERSONAL STUDY

Study of pregnancy progression, birth outcomes, and the health of the fetus and newborn in women without rh negative isosensitization.

The clinical, laboratory, and functional indicators of 64 out of 77 Rh-negative (Rh-) pregnant women (83.1%) without Rh isoimmunization were examined. In 13 women (16.9%), moderate to severe Rh isoimmunization was identified.

The average age of the Rh-negative pregnant women without isoimmunization (n = 64) was 30.55 ± 7.0 years, with a range of 18 to 43 years. The somatic and obstetric history of these 64 women was analyzed. Among them, 24 (37.5%) were primiparous, while 40 (62.5%) were multiparous.

In the obstetric history of the 40 multiparous Rh-negative women without isoimmunization, the average number of pregnancies was 2.34 ± 0.6 (ranging from 1 to 5).

Their reproductive histories showed the following distribution: 55.4% had previous births, 25.7% had miscarriages, 9.5% had elective abortions, 6.8% had spontaneous miscarriages, and 2.7% had ectopic pregnancies.

Pregnancy complications in Rh-Negative women without isoimmunization:

First trimester: 27.3% experienced anemia, 21.2% early toxicosis, 17.4% asymptomatic bacteriuria, and 14.4% were diagnosed with a threat of miscarriage.

Second trimester: 28.6% developed anemia, 21.4% had asymptomatic bacteriuria, 15.7% faced a threat of miscarriage, 12.9% had an exacerbation of chronic pyelonephritis, and 10% experienced mild preeclampsia.

Third trimester: 22.6% were diagnosed with a risk of uterine rupture, 18.5% with a threat of premature birth, and 16.9% with acute respiratory viral infection (ARVI).

Prophylactic administration of anti-D immunoglobulin was carried out at an average gestational age of 28.7 ± 0.09 weeks. Among women without Rh isoimmunization, the cesarean section

rate was 73.4%, while 26.52% delivered vaginally. The overall rate of preterm birth (via both cesarean section and vaginal delivery) was 15.62%.

Among the indications for cesarean section (73.4%), the distribution was as follows: 49.1% due to a uterine scar, 14% due to acute fetal hypoxia on the background of chronic hypoxia, 10.5% due to varicose veins of the external genitalia, 8.8% due to breech presentation, and 7% in pregnancies conceived via assisted reproductive technologies. 75% of newborns were delivered in satisfactory condition, 18.8% in moderate to severe condition, and 6.3% in severe condition. An uncomplicated early neonatal period was observed in 62.3% of newborns. The remaining 37.7% presented with the following complications: chronic fetal hypoxia (10.4%), respiratory distress syndrome (14.3%), morphofunctional immaturity (5.2%), cerebral circulation disorders of grade I–II (5.2%), and signs of intrauterine infection (2.6%).

Based on the study results, administration of anti-D immune-globulin during pregnancy in Rh-negative women is an effective measure for the prevention of Rh incompatibility.

Frequency of risk factors for Rh isoimmunization

In the study, Rh isoimmunization was identified in 13 out of 77 Rh-negative (Rh^-) pregnant women, accounting for 16.9% of the total group. The mean age of the women with isoimmunization was 30.3 ± 4.16 years, with an age range primarily between 25 and 41 years. An analysis of obstetric history revealed that the average age at first sexual intercourse was 23.26 ± 2.5 years (range: 17–28). On average, these women had experienced 4.0 ± 2.2 pregnancies (range: 1–10), with 2.2 ± 0.9 births (range: 1–4), and 2.0 ± 1.7 abortions (range: 1–5). The average number of home deliveries was 1.1 ± 0.3 (range: 1–2).

The study also examined the presence and frequency of various risk factors associated with Rh isoimmunization among the affected pregnant women.

As a result of the study, it was determined that

12 out of 13 (92.3%) pregnant women with isosensitization had previously given birth. Among these patients, 11 (84.6%) had not received anti-D immunoprophylaxis after a previous miscarriage.

Additionally, 7 women (53.8%) had a history of antenatal fetal death, 6 (46.2%) had experienced partial placental abruption in a previous pregnancy, 6 (46.2%) had bleeding during the first trimester of a previous pregnancy, 4 (30.8%) had a history of induced abortions, 2 (15.4%) had a spontaneous miscarriage, and 2 (15.4%) had an incorrectly determined Rh status.

Features of pregnancy in women with rh isoimmunization

In this study, Rh isoimmunization was observed during pregnancy in 13 out of 77 (or 16.9%) Rh-negative women. The course of the current pregnancies was analyzed, and the incidence of complications was determined. The risk of nervous system tension in pregnant women with Rh isoimmunization was found to be quite high, approximately 23.8%. The probability of anemia was 19%. Additionally, asymptomatic bacteriuria was detected in 16.7% of cases, the threat of miscarriage in 14.3%, and early toxicosis in 11.9%. A positive indirect Coombs test was found in 7.1% of pregnant women in the first trimester.

During the first trimester, nervous system tension was noted in 10 out of 13 women with isoimmunization, representing 76.9% of the study group. Anemia was present in 61.5%, or 8 women, while asymptomatic bacteriuria was observed in 7 women (53.8%). In addition, 6 (46.2%) of the pregnant women were diagnosed with a threat of miscarriage, 5 (38.5%) with early toxicosis, 3 (23.1%) with acute respiratory viral infections, and 3 (23.1%) with a positive indirect Coombs test.

The incidence of complications during the second trimester was assessed, and 10 out of 13 pregnant women with isoimmunization (76.9%) exhibited signs of HDF. Anemia was detected in 5 women, representing 38.5% of the study group, while laboratory signs of asymptomatic bacteriuria were observed in 4 women, or 30.8% of the participants.Upon analyzing the signs of HDF, it was determined that ultrasound and Doppler signs were detected on average at 25.1 ± 1.3 weeks of pregnancy (range: 23–27 weeks). The incidence of ultrasound and Doppler signs of HDF during the second trimester is presented in Table 1.

Table 1

Frequency of ultrasound and doppler signs of HDF in the second trimester of gestation

Signs of HDF		**%**
Ultrasound Findings (USM):		
Increased pericardial fluid	10	00
Hepatosplenomegaly	10	00
Cardiomegaly and dilation of all heart chambers	10	00
Polyhydramnios	10	00
Placentomegaly	10	00
Edema	8	80
Ascites	7	70
Based on Dopplerography		
Peak systolic blood flow velocity in the middle cerebral artery, >1.5 MoM	10	00

As shown in the table, 10 out of 13 women with isosensitization (76.9%) exhibited signs of HDF during the second trimester. These signs included increased pericardial fluid (100%), hepatosplenomegaly (100%), cardiomegaly and dilation of all heart chambers (100%), polyhydramnios (100%), placentomegaly (100%), edema (80%), and ascites (70%).

Doppler examination revealed that the maximum systolic blood flow velocity in the middle cerebral artery was greater than 1.5 MoM in all 10 fetuses (100%).

In pregnant women with isosensitization, HDF, anemia, AFD, the risk of preterm birth, and mild preeclampsia were more frequently observed in the third trimester. Clinical signs of HDF appeared at an average of 30.3±1.2 weeks (range 29-32 weeks).

It is important to note that all 13 women with Rh isosensitization showed HDF, 4 had anemia, 3 had AFD, 2 had the threat of premature birth, and 2 had mild preeclampsia.

Therefore, among pregnant women with Rh isosensitization:

In the first trimester, 76.9% exhibited nervous system tension, 61.5% had anemia, 23.3% had asymptomatic bacteriuria, 46.2% were at risk of miscarriage, 38.5% had early toxicosis, 23.1% had Acute respiratory viral infections, and 23.1% had a positive indirect Coombs test.

In the second trimester, 76.9% showed HDF, 38.5% had anemia, and 30.8% had asymptomatic bacteriuria.

In the third trimester, clinical and laboratory signs of HDF were present in all patients (100%), with 30.8% showing anemia, 23% having AFD, 15.4% at risk for premature birth, and 15.4% with mild preeclampsia.

Clinical and laboratory signs of HDF were detected on average at 25.1±1.3 weeks (range 23-27 weeks) during the second trimester, and at 30.3±1.2 weeks (range 29-32 weeks) during the third trimester.

Results of ultrasound examinations in pregnant women with hemolytic disease of the fetus

The echographic findings of pregnant women with Rh isosensitization and HDF were compared with those of women with uncomplicated pregnancies.

Upon analyzing the echographic indicators of HDF, it was found that during the second trimester of pregnancy, the disease manifested as a statistically significant increase in the echographic parameters: placental thickness (40.9±1.87 mm), fetal liver length (34.1±0.9 mm), spleen length (35.7±0.21 mm), the ratio of fetal heart circumference to chest circumference (0.58±0.03), and the amniotic fluid index (25.1±0.03 cm) ($P<0.05$). Additionally, edema, ascites, double contour of the fetal scalp, edema of the umbilical cord, and dilatation of the umbilical veins were detected. In the third trimester, a statistically significant increase was observed in placental thickness (44.3±0.11 mm), fetal liver length (55.4±0.16 mm), spleen length (41.2±0.9 mm), and the amniotic fluid index (17.1±0.21 cm) in pregnant women with HDF ($P<0.05$). At the same time, the fetus exhibits edema, ascites, a double contour of the fetal scalp, umbilical cord edema, and prominent visualization of the veins with dilation.

In pregnant women with HDF during the third trimester, a statistically significant increase was observed in placental thickness

(44.3±0.11 mm), fetal liver length (55.4±0.16 mm), spleen length (41.2±0.9 mm), and the amniotic fluid index (17.1±0.21 cm) (P<0.05).

Following pathogenetic treatment (intrauterine hemotransfusion), a reduction in pericardial fluid and umbilical cord edema was noted in the fetus, along with the resolution of fetal edema, ascites, and the absence of double contours of the scalp.

Results of doppler examination in pregnant women with hemolytic disease of the fetus

The results of the resistance index (RI), pulsatility index (PI), and average blood flow velocity (V) in the fetal umbilical cord during pregnancy are presented in Table 2. Additionally, the measurements of the fetal MCA-PSV were assessed.

Table 2

Dopplerographic indicators during pregnancy in women with rhesus isoimmunization

Dopplerographic indicators	Pregnancy dynamics	
	II trimester	III trimester
RI	0,64±0,03 (0,63-0,65)	0,60±0,04 (0,59-0,62)
PI	1,09±0,02 (1,08-1,1)	1,0±0,04 (0,9-1,1)
V	26,2±0,06 (25,3-27,0)	29,8±0,1 (28,7-30,0)
MCA-PSV, cm/s (>1,5 MoM),	71,63±3,59 (48,36-85)	80,2±2,15 (71,3-97)

As shown in the table, during pregnancy in women with Rh isosensitization, the indicators of RI, PI, and V in the umbilical cord did not differ from their physiological parameters. However, the MCA-PSV indicators significantly increased and were above 1.5 MoM.

Results of invasive diagnostic methods for hemolytic disease of the fetus

Invasive methods were used to diagnose HDF in the study.

Amniocentesis and cordocentesis were performed on all pregnant women with Rh isosensitization (n=13) as part of the invasive prenatal diagnostic methods.

Indications for Amniocentesis, Technique, and Results

Amniocentesis was performed in the second trimester of pregnancy at 25.1±1.35 weeks and in the third trimester at 30.3±1.2 weeks.

In pregnant women with isosensitization, amniocentesis in the second trimester of gestation (n=10) was indicated based on echographic and Dopplerographic indicators of HDF . These included increased placental thickness (40.9±1.87 mm), increased fetal liver length (34.1±0.9 mm), increased fetal spleen length (35.7±0.21 mm), an increased ratio of fetal heart circumference to thoracic circumference (0.58±0.03), an increased amniotic fluid index (AMI) (25.1±0.03 cm), edematous fetus, presence of ascites, double contour of the fetal scalp, edema and dilation of umbilical veins, and increased peak systolic blood flow velocity in the middle cerebral artery (>1.5 MoM; 71.63±3.59 cm/s).

Amniocentesis was also performed in the third trimester of pregnancy according to the same indications (n=3).

The karyotype of the fetus was determined from the amniotic fluid obtained via amniocentesis, and chromosomal aberrations were excluded. The karyotype was 46,XY and 46,XX.

Additionally, a microbiological study of the amniotic fluid was performed using polymerase chain reaction (PCR). Cytomegalovirus (CMV), parvovirus (P19), and Toxoplasma were analyzed, and no infections were detected.

Indications for and technical features and outcomes of cordocentesis, an invasive diagnostic method for fetal hemolytic disease

Cordocentesis was utilized as an invasive method for diagnosing and providing pathogenetic treatment of fetal hemolytic disease, specifically for intrauterine hemotransfusion of the fetus.

Hemoglobin (Hb), hematocrit (Hct), and platelet count were measured in the fetal blood obtained through cordocentesis. The results are presented in Table 3.

Table 3.

Laboratory indicators of blood obtained through cordocentesis in HDF

Blood indicators	Results obtained	Normative indicators Indicators
Hb, (g/dl)	3,11±0,56 (0,5-7,2)	11-15
Hct, (%)	9,85±1,97 (2,6-22,8)	33-45
Plt, (10^9/l)	118,44±23,1 (33-212)	187-274

Note: The normative indicators of laboratory blood tests were taken from K.N. Nicolaides.

As shown in the table, a significant decrease in hemoglobin (Hb), hematocrit (Hct), and platelet count (PLT) is observed in HDF. In HDF, as assessed through the invasive method of cordocentesis, there is a marked reduction in Hb (3.11±0.56 g/dl), Hct (9.85±1.97%), and PLT (118.44±23.1 x 10^9/L).

Indications for intrauterine blood transfusion, technique, effectiveness, and effects on the fetus and mother

The decision to perform an intrauterine hemotransfusion was made based on the results of laboratory tests. The procedure was carried out under sterile conditions with ultrasound guidance. To reduce fetal movement, a paralytic drug, vecuronium (0.01 mg/kg), was injected into the umbilical vein, considering the fetal position. Immediately afterward, blood transfusion was initiated.

The blood volume was calculated using the formula provided by Moise (1997): Based on the formula:

$$V = \frac{FPH((Hdf)\ Hct - (Ptf)\ Hct)(Db)Hct}$$

Where: *(Hdf) Hct* – Target Hct, *(Ptf) Hct*– Pretransfusion Hct, *(Db) Hct* – Donor blood Hct.

Fetoplacental Blood Volume (FBV) is determined using the formula:

$$FBV=1.046+(0.14\times EWFU)$$

Where: *EWFU* - estimated weight of the fetus on ultrasound.

A total of 27 intrauterine hemotransfusions were performed. Of the 13 pregnant women, 13 (100%) received one transfusion, 8 (61.1%) received two transfusions, and 6 (46.2%) received three transfusions.

The average volume of the first hemotransfusion was 117.92±13 (70-200) ml, the second was 138.63±11.5 (100-180) ml, and the third was 128.63±18.1 (83-200) ml. During the procedure, hemoglobin and hematocrit levels of the donor blood were measured. The laboratory analyses of the donor blood are presented in Table 4.

Table 4

Laboratory analyses of donor blood used in intrauterine Hemotransfusions

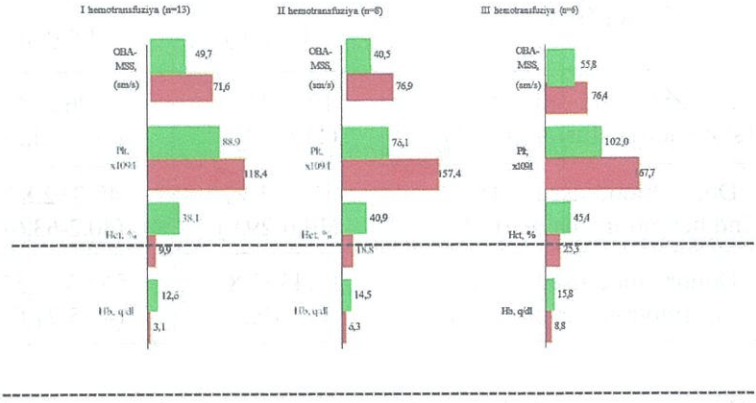
Donor Blood	Results of Laboratory Analysis	
	Hb (q/dl)	Hct (%)
Donor blood used in the 1st hemotransfusion (n=13)	17,54±0,9 (11,6-22,2)	71,96±2,68 (34,8-64,2)
Donor blood used in the 2nd hemotransfusion (n=8)	18,5±1,27 (14,6-29,9)	49,3±2,82 (40,2-63,6)
Donor blood used in the 3rd hemotransfusion (n=6)	19,43±1,87 (17-19,5)	56,63±4,55 (43,5-71,8)

During the transfusion, the position of the needle in the umbilical vein was continuously monitored using ultrasound transducers throughout the procedure. Additionally, fetal myocardial contractility was visualized, taking into account the potential risk of hypervolemic shock in the fetus. The average duration of the intrauterine hemotransfusion was 45.0±2.85 minutes. After the transfusion, 4 ml of fetal blood was collected and sent for laboratory analysis, and the puncture needle was removed from the umbilical vein under ultrasound guidance. Pregnant women who underwent intrauterine

blood transfusion were monitored in a hospital setting for 1-3 days. Their general condition, blood pressure, pulse, and fetal movements were egularly assessed.

After each hemotransfusion, the maximum systolic blood flow velocity (MCA-PSV) in the middle cerebral artery was evaluated through laboratory blood tests and Doppler ultrasound.

After the second intrauterine hemotransfusion, a significant increase in hemoglobin (Hb) and hematocrit (Hct) levels, along with a decrease in platelet count (PLT) and MCA-PSV, was observed ($P<0.05$).A similar trend was noted after the third intrauterine hemotransfusion: a statistically significant increase in Hb and Hct levels and a notable decrease in MCA-PSV ($P<0.05$). The changes in Hb, Hct, PLT, and MCA-PSV before and after intrauterine hemotransfusions in the study are illustrated in Graph 1.



Graph 1. Characteristics of changes in Hb, Hct, PLT, MCA-PSV before and after intrauterine hemotransfusions in the conducted study

In the study, the interval between intrauterine hemotransfusions was determined. The results obtained are presented in Table 5.

Table 5.

Gestation period of Intrauterine hemotransfusions in HDF in pregnant women with isoimmunization

Intrauterine hemotransfusion	Gestation period
First Intrauterine Fetal hemotransfusion for (n=13)	26,11±0,78 (22,5-32)
Second Fetal Intrauterine Hemotransfusion (n=8)	28,15±0,86 (24,5-32)
Third Intrauterine Fetal Hemotransfusion (n=6)	31,67±0,64 (30-33,6)

The fetus received repeated intrauterine hemotransfusions at 2-3 week intervals. The results of laboratory analyses of fetal blood before the second and third hemotransfusions were compared. The findings are presented in Table 6.

Table 6

Characteristics of changes in laboratory analyses after intrauterine hemotransfusions

Intrauterine hemotransfusions	Hb, q/dl	Hct, %	PLt x10^9/l	MCA-PSV, cm/s
Before Fetal hemotransfusion I	3,11±0,56 (0,5-7,2)	9,85±1,97 (2,6-22,8)	118,44±23,1 (33-212)	71,63±3,5 9 (48,36-97)
Before Fetal hemotransfusion II	6,3±0,87 (3,4-9,6)	18,79±1,6 6 (10-29,1)	157,43±20,6 (89-283)	76,89±7,1 9 (47-107)
Before Fetal hemotransfusion III	8,75±0,91 (4,8-11,5)	25,25±2,5 8 (13,6-30,9)	167,67±38,4 9 (77-292)	76,4±50 2 (98,75)
P	P1-2<0,05; P2-3>0,05; P1-3<0,05	P1-2<0,05; P2-3>0,05; P1-3<0,05	P1-2>0,05; P2-3>0,05; P1-3>0,05	P1-2>0,05; P2-3>0,05; P1-3>0,05

Thus, intrauterine hemotransfusion is highly effective in treating hemolytic disease of the fetus. This is manifested by a statistically significant increase of Hb (12.61±0.95 g/dl), Hct (38.08±3.29 %), and a decrease of MCA-PSV (49.65±2.4 cm/s) after the first hemotransfusion, an increase of Hb (14.49±1.22 g/dl), Hct

(40.92±3.63 %), a decrease of Plt (76.14±14.33 x 10^9/l) and MCA-PSV (40.53±3.98 cm/s) after the second hemotransfusion, and a statistically significant increase of Hb (15.8±0.59 g/dl) and Hct (45.4±1.89 %), and a decrease of MCA-PSV (55.77±3.1 cm/s) after the third hemotransfusion. ($P<0.05$).

The effect of intrauterine hemotransfusions on the condition of newborns and perinatal outcomes

In the study, 13 fetuses were born after intrauterine hemotransfusion in women with isosensitization. The births occurred at a mean gestational age of 33.44±1.5 (25.6-37) weeks. Intrauterine death was observed in 3 (23.1%) of the 13 newborns. The average gestational age of the deceased fetuses was 26.3±1.3 (24-29) weeks. Among the deceased fetuses, two received 1 intrauterine hemotransfusion, and one received 2. One of the three fetuses died within 72 hours after the intrauterine hemotransfusion due to developmental defects, while two others died as a result of transient and persistent bradycardia.

Thus, antenatal death was recorded in 3 (23.1%) fetuses who underwent intrauterine hemotransfusion.

The average birth weight of the 10 newborns born alive was 2383.85±152.4 (1440-3300) g.

Of the 10 live-born newborns, 6 (60%) were delivered by cesarean section at 36.6±0.47 weeks of gestation, while 4 (40%) were delivered vaginally. Vaginal deliveries occurred at 35.0±1.2 (34-37) weeks of gestation.

Regarding the condition of the newborns after delivery: 20% (n=2) were born in an adequate condition 60% (n=6) were born in moderate to severe condition 20% (n=2) were born in a severe condition.

In the early neonatal period, the following conditions were diagnosed in the newborns: Hemolytic disease (100%) respiratory distress syndrome (80%) Morphofunctional insufficiency (50%) Encephalopathy (30%) Pneumonia (10%).

As a result of the study, hemolytic disease of varying degrees was detected in all 10 newborns (100%). Of these: 2 (20%) were anemic 6 (60%) had jaundice; 2 (20%) had edema.

The average number of hemotransfusions performed on the newborns was 2.38±0.095 (range: 1-5). The duration of their treatment in the intensive care unit was 14.3±11.9 (range: 1-40) days. All newborns were discharged home in adequate condition. Following discharge, the newborns were regularly monitored by a pediatrician, hematologist, and neuropathologist throughout their lives. Their physical and intellectual development was virtually identical to that of healthy newborns.

RESULTS

1. In women without Rh-negative isosensitization, pregnancy:

- First trimester: Anemia was observed in 27.3%, early toxicosis in 21.2%, asymptomatic bacteriuria in 17.4%, and threatened miscarriage in 14.4%.

- Second trimester: Anemia was noted in 28.6%, asymptomatic bacteriuria in 21.4%, threatened miscarriage in 15.7%, exacerbation of chronic pyelonephritis in 12.9%, and mild preeclampsia in 10%.

- Third trimester: The threat of uterine rupture was present in 22.6%, the threat of premature birth in 18.5%, and acute respiratory viral infection in 16.9%. In women without Rh-negative isosensitization, preventive immunization with anti-D immunoglobulin at 28.7±0.9 weeks of gestation is mandatory.

- In women without Rh-negative isosensitization:

The frequency of cesarean section was 73.4%, while natural births occurred in 26.6%. The frequency of premature births was 15.6%. Indications for cesarean section included a uterine scar in 49.1%, acute fetal hypoxia in 14%, varicose veins of the external genitalia in 10.5%, breech presentation in 8.8%, and pregnancy following in vitro fertilization in 7%. It was found that 75% of newborns were in good condition, 18.8% were in moderate condition, and 6.3% were in severe condition. 62.3% of newborns were born without complications, while 37.7% had conditions resulting from chronic fetal hypoxia, 10.4% had respiratory distress syndrome, 5.2% exhibited morphofunctional immaturity, 5.2% had first and second-degree cerebral circulation disorders, and 2.6% had

intrauterine infections [3,9,10,11,12].

2. Factors contributing to Rh isosensitization include: 92.3% of cases were associated with previous births, 84.6% after a miscarriage, 53.8% after antenatal fetal death, 46.2% with premature partial separation of the placenta in a previous pregnancy, 46.2% with vaginal bleeding, 30.8% had a history of induced abortions, 15.4% had spontaneous miscarriages, and 15.4% involved incorrect determination of the mother's Rh status [1,2].

3. In women with Rh sensitization:

- In the first trimester of pregnancy: 76.9% experienced nervous system tension, 61.5% had anemia, 23.3% had asymptomatic bacteriuria, 46.2% had a threatened miscarriage, 38.5% had early toxicosis, 23.1% had acute respiratory viral infection, and 23.1% had a positive indirect Coombs test.

- In the second trimester of pregnancy: 76.9% had fetal hemolytic disease, 38.5% had anemia, and 30.8% had asymptomatic bacteriuria.

- In the third trimester of pregnancy: 100% of patients exhibited clinical and laboratory signs of fetal hemolytic disease, 30.8% had anemia, 23% experienced antenatal fetal death, 15.4% faced a threat of premature birth,and 15.4% had mild preeclampsia.

Clinical, laboratory, and functional signs of fetal hemolytic disease were observed at an average of 25.1±1.3 weeks in the second trimester and at 30.3±1.2 weeks in the third trimester[7].

4. Echographic criteria for fetal hemolytic disease:

In the second trimester, a statistically significant increase in the following echographic parameters was observed: Placental thickness: 40.9±1.87 mm, Fetal liver length: 34.1±0.9 mm, Fetal spleen length: 35.7±0.21 mm, Ratio of fetal heart circumference to chest circumference: 0.58±0.03, Amniotic fluid index: 25.1±0.03 cm. Additionally, edema, ascites, double contour of the fetal scalp, edema of the umbilical cord, and dilatation of the umbilical veins were prominently visualized in the fetus.

In the third trimester of pregnancy, a statistically significant increase in the following echographic parameters was observed: Placental thickness: 44.3±0.11 mm, Fetal liver length: 55.4±0.16 mm, spleen length: 41.2±0.9 mm, Amniotic fluid index: 17.1±0.21 cm ($P<0.05$).

After intrauterine hemotransfusion, the following improve-

ments were observed in the fetus: A decrease in pericardial fluid, Reduction of umbilical edema, Absence of fetal edema and ascites, absence of the double contour of the fetal head.

In Rh-negative women, an increase of the peak systolic velocity of the middle cerebral artery to 71.63±3.59 cm/s in the second trimester and 80.2±2.15 cm/s in the third trimester (>1.5 MoM) serves as a non-invasive diagnostic criterion for fetal hemolytic disease [5,6].

5. Invasive diagnostic methods for fetal hemolytic disease: Amniocentesis revealed no presence of cytomegalovirus, parvovirus, or toxoplasma in the amniotic fluid. Additionally, no chromosomal aberrations were detected through genetic examination. Cordocentesis for the diagnosis of fetal hemolytic disease showed a significant decrease in the following blood parameters: Hemoglobin (Hb): 3.11±0.56 g/dl, Hematocrit (Hct): 9.85±1.97%, Platelet count (PLT): 118.44±23.1 x $10^{\wedge 9/L}$ [4,14].

6. Intrauterine hemotransfusion has proven to be highly effective in treating fetal hemolytic disease. Following the first hemotransfusion, hemoglobin levels increased by 12.61±0.95 g/dL and hematocrit by 38.08±3.29%, accompanied by a 49.65±2.4 cm/s reduction in the peak systolic velocity of the middle cerebral artery. After the second hemotransfusion, hemoglobin rose to 14.49±1.22 g/dL, hematocrit reached 40.92±3.63%, the MCA peak systolic velocity decreased by 40.53±3.98 cm/s, and platelet count dropped by 76.14±14.33×10^9/L. Following the third hemotransfusion, hemoglobin increased to 15.8±0.59 g/dL and hematocrit to 45.4±1.89%, along with a significant change in the peak systolic velocity of the MCA ($P<0.05$).

Of the newborns born after intrauterine hemotransfusion, 20% were born in adequate condition, 60% in moderate-to-severe condition, and 20% in severe condition. In the early neonatal period, all newborns (100%) were diagnosed with hemolytic disease of varying severity, 80% with respiratory distress syndrome, 50% with morphofunctional immaturity, 30% with encephalopathy, and 10% with pneumonia. The average number of replacement hemotransfusions performed on newborns during the early neonatal period was 2.38±0.095. in the intensive care unit was 14,3±11,9 days. All

newborns were discharged home in adequate condition. The main and most effective treatment for fetal hemolytic disease in women with Rh isosensitization is intrauterine hemotransfusion. A significant decrease in antenatal mortality rates, from 100% to 23.1%, was observed [13,14].

PRACTICAL RECOMMENDATIONS

1. In Rh-negative women, preventive immunization with anti-D immunoglobulin is mandatory after childbirth, following a miscarriage, in cases of bleeding during pregnancy, spontaneous abortion, ectopic pregnancy, antenatal fetal death, and after invasive procedures during pregnancy.
2. For Rh-negative women without isosensitization, a dose of 300 mcg (1500 IU) should be administered at 28.7±0.9 weeks of pregnancy and within 72 hours after delivery (if the newborn is Rh-positive), preferably within the first 2 hours.
3. In pregnant women with Rh isosensitization, clinical, laboratory, and functional evaluations, including ultrasound and Doppler examination, should be conducted in the early stages of pregnancy. Regardless of the indirect Coombs test results, in the second trimester, placental enlargement, fetal liver and spleen enlargement, increased amniotic fluid, increased heart-to-chest circumference ratio, the presence of gallbladder, ascites, double contour of the head, dilation of the umbilical veins, and increased peak systolic velocity of the middle cerebral artery according to Doppler (>1.5 MoM) are indicative of fetal hemolytic disease and warrant invasive diagnostic methods.
4. In fetal hemolytic disease, genetic and microbiological testing of amniotic fluid obtained by amniocentesis is feasible. Detection of cytomegalovirus, parvovirus, and toxoplasma in the amniotic fluid allows for the diagnosis of intrauterine infections in the fetus.
5. In hemolytic disease of the fetus, it is essential to assess hemoglobin, hematocrit, and platelet levels via cordocentesis, and intrauterine hemotransfusion remains the primary and most

effective treatment.

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LIST OF ABBREVIATIONS

ABO	– blood group
AFI	– amniotic fluid index
CMV	– cytomegalovirus
AFD	– antenatal fetal death
IHF	– intrauterine hemotransfusion of the fetus
HDF	– hemolytic disease of the fetus
FBV	– Fetoplacental Blood Volume
Hb	– Hemoglobin
Hct	– hematocrit
ICD	– International Classification of Diseases– in vitro fertilisation
MCA-PSV	– peak systolic blood flow velocity in the middle cerebral artery
PI	– pulsatility index
P19	– parvovirus
PCR	– polymerase chain reaction
RI	– resistance index

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