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Original Research Article

Perinatal outcomes and hematologic parameters of neonates born to Rh-negative mothers with and without isoimmunization

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ABSTRACT

Background: Rh isoimmunization remains a significant cause of neonatal morbidity and mortality in Rh-negative pregnancies. It causes hemolytic disease of the fetus and newborn (HDFN), leading to anemia, hyperbilirubinemia, and perinatal complications. This study compares hematologic parameters and perinatal outcomes of neonates born to Rh-negative mothers with and without isoimmunization.
Methods: This cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Mymensingh Medical College Hospital, Bangladesh, from July 2019 to December 2019. Eighty Rh-negative pregnant women were enrolled, comprising five isoimmunised and seventy-five non-isoimmunised mothers. Data on neonatal haemoglobin, serum bilirubin, direct Coombs test results, Apgar scores, and treatment requirements were collected. Maternal factors were documented, including gravidity, antenatal care, and anti-D prophylaxis. Statistical analyses used SPSS version 25.0, with p-values <0.05 considered significant.
Results: Neonates of isoimmunised mothers had lower haemoglobin levels (mean <12 g/dl in 60% vs. 0%, p<0.001), elevated bilirubin ≥4 mg/dl (80% vs. 20%, p<0.001), and 100% direct Coombs test positivity compared to none in non-isoimmunised neonates. Phototherapy and exchange transfusion were required in 80% and 60% of isoimmunised neonates, significantly higher than the non-isoimmunised group. Poor Apgar scores (<6 at 5 minutes) were more frequent in isoimmunised neonates (40% vs. 12%). High gravidity, inadequate antenatal care, and absent anti-D prophylaxis were prevalent among isoimmunised mothers.
Conclusion: Rh isoimmunization markedly worsens neonatal hematologic and perinatal outcomes. Strengthened antenatal screening, universal anti-D prophylaxis, and enhanced neonatal care are critical to reducing HDFN burden in at-risk populations.

Keywords: Rh isoimmunization, Hemolytic disease of the newborn, Neonatal anemia, Hyperbilirubinemia, Perinatal outcomes

INTRODUCTION

Rh incompatibility is one of the most important preventable causes of perinatal morbidity and mortality globally. It occurs when a Rh-negative mother is pregnant with a Rh-positive fetus, usually due to the paternal genotype. The following immunologic reaction can result in the production of anti-D antibodies that cross the placenta, causing hemolysis of fetal red blood cells—a condition termed hemolytic disease of the fetus and newborn (HDFN).[1]

The Rh blood group system, discovered by Landsteiner and Weiner in 1941, consists of over 49 antigens, among which the D antigen is the most immunogenic.[2] The gene encoding the Rh antigens is located on chromosome 1 and is inherited in an autosomal dominant manner.[3] The frequency of Rh negativity varies globally: it is highest among Basques (34%) and lowest among Asians (1%). In Bangladesh, the prevalence is about 5.5%.[4]

The pathophysiology of Rh isoimmunization involves maternal sensitization due to exposure to Rh-positive red cells through fetomaternal hemorrhage, miscarriage, abortion, or invasive obstetric procedures. The initial IgM response cannot cross the placenta, but subsequent IgG responses can, leading to fetal red cell destruction.[5] The severity of hemolysis depends on the volume of fetomaternal hemorrhage and the antibody titer.[6] Conditions such as hydrops fetalis, severe anemia, hyperbilirubinemia, and neonatal death can result if untreated.[1]

Prophylactic administration of anti-D immunoglobulin has significantly reduced the incidence of Rh sensitization. According to ACOG guidelines, prophylaxis is recommended at 28 weeks of gestation and within 72 hours postpartum or after any potentially sensitizing event.[7] The incidence of Rh isoimmunization in Rh-negative mothers who are also ABO incompatible is reduced dramatically to 1-2%. This is believed to occur because the mother's serum contains antibodies against the foetus's ABO blood group.[8] Surprisingly, about 30% of Rhesus-negative individuals never become sensitized as they are immunologically non-responders.[9]

In a Rh-negative mother, there is an increased incidence of pre-eclampsia, polyhydramnios, placentomegaly, a big baby with its hazards, hypofibrinogenemia due to prolonged retention of a dead fetus in utero, PPH due to a big placenta and blood coagulopathy, and Maternal syndrome, which manifests with generalized oedema, proteinuria, and pruritis due to cholestasis.[1] The clinical manifestation of Rh disease ranges from mild anemia to severe hydrops. According to Bowman's classification, mild disease affects 50% of sensitized fetuses and often requires no treatment, while moderate disease may lead to kernicterus. Severe forms account for up to 25% of cases and may result in intrauterine death or early neonatal mortality.[10]

Coombs testing (direct and indirect) is essential in identifying affected neonates. A positive direct Coombs test (DCT) confirms antibody-coated erythrocytes, indicating ongoing hemolysis. Cord blood analysis for haemoglobin and bilirubin levels provides critical insight into the severity of hemolytic anemia and guides interventions such as phototherapy or exchange transfusion.[11]

Several studies support the increased risk of hematologic abnormalities in neonates of isoimmunised mothers. Shradha et al. observed significantly higher rates of hyperbilirubinemia, anemia, and need for exchange transfusion in infants born to Coombs-positive mothers compared to those born to Coombs-negative mother.[12] Similarly, Eleje et al found a high incidence of neonatal jaundice and perinatal mortality among isoimmunised pregnancies in Nigeria.[13]

While developed countries have controlled mainly Rh disease through rigorous antenatal screening and prophylaxis programs, the burden remains high in developing nations. Nagamuthu et al and Khatun et al. reported significant rates of Rh isoimmunization-related neonatal complications in South Asian populations, including anemia, jaundice, hydrops fetalis, and death.[14,15]

This study aims to evaluate and compare the hematologic and perinatal outcomes among neonates born to Rh-negative mothers with and without isoimmunization. Focusing on haemoglobin levels, bilirubin concentrations, and Coombs test results, it provides insight into the clinical burden of Rh sensitization and underscores the importance of preventive strategies in routine obstetric care.

Objective

This study aimed to compare the neonatal hematologic parameters and perinatal outcomes of Rh-negative mothers with and without isoimmunization.

METHODS

This cross-sectional, descriptive observational study was conducted at the Department of Obstetrics and Gynaecology, Mymensingh Medical College Hospital, Mymensingh, Bangladesh, from July 2019 to December 2019. Based on predefined eligibility criteria, 80 Rh-negative pregnant women were included in the study.

Sample selection

Participants were selected purposively from admitted Rh-negative pregnant women who fulfilled the inclusion criteria. The participants were divided into two groups.

Isoimmunized group (n=5)

Rh-negative mothers with a positive indirect Coombs test (ICT).

Non-isoimmunized group (n=75)

Rh-negative mothers with negative ICT.

Inclusion criteria

Rh-negative pregnant women admitted after 28 weeks of gestation. Singleton pregnancy. Cord blood analysis is available for haemoglobin, bilirubin, and Coombs tests. Willingness to participate and provide informed consent.

Exclusion criteria

Gestational age below 28 weeks. Multiple gestation pregnancies. Mothers with pre-existing hematologic disorders. Cases with incomplete data or where cord blood sampling was not feasible.

Ethical approval

Ethical approval for this study was obtained from the institutional review board of Mymensingh Medical College. Written informed consent was taken from each participant after explaining the study's purpose, procedures, and voluntary nature. Data were collected using a structured and pre-tested case record form. Maternal history, antenatal Coombs test results, and delivery-related information were recorded. At birth, cord blood samples were collected to assess haemoglobin concentration, total serum bilirubin, and direct Coombs test results.

Neonatal outcomes were documented, including Apgar scores, need for phototherapy, exchange transfusion, and NICU admission. All data were collected by trained personnel to ensure accuracy and consistency. Participants retained the right to withdraw at any time without consequence. Confidentiality and anonymity were strictly maintained throughout. Statistical analysis was performed using SPSS version 25.0. Descriptive statistics were used to summarize variables, while independent t-tests and Chi-square or Fisher's exact tests were applied to assess group differences. A p value <0.05 was considered statistically significant.

RESULTS

Table 1 presents maternal characteristics associated with isoimmunization risk. Among the five isoimmunized mothers, 80% were multigravida (≥3), and all had inadequate or no history of anti-D prophylaxis. Four had poor antenatal follow-up, and three had a history of previously affected fetuses.

In contrast, these risk factors were far less prevalent in the non-isoimmunized group. Cesarean delivery was the most common delivery mode in both groups. These maternal patterns provide insight into the underlying causes of isoimmunization and contextualize the adverse neonatal outcomes observed in this study.

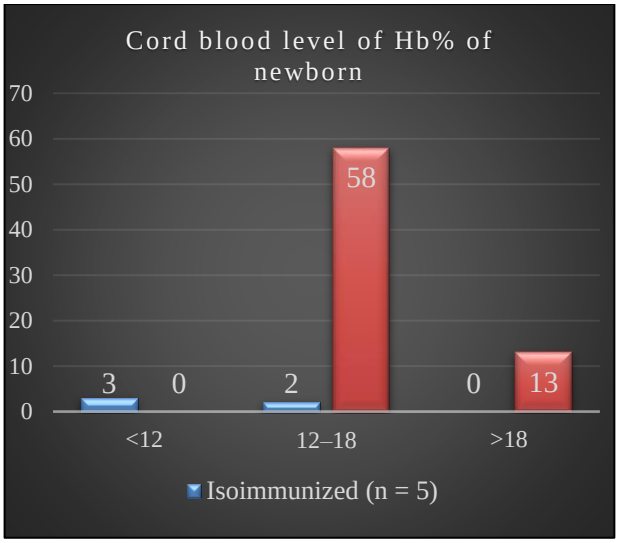

Figure 1: Cord blood level of Hb% of newborns in isoimmunized and nonimmunized pregnancies (n=80).

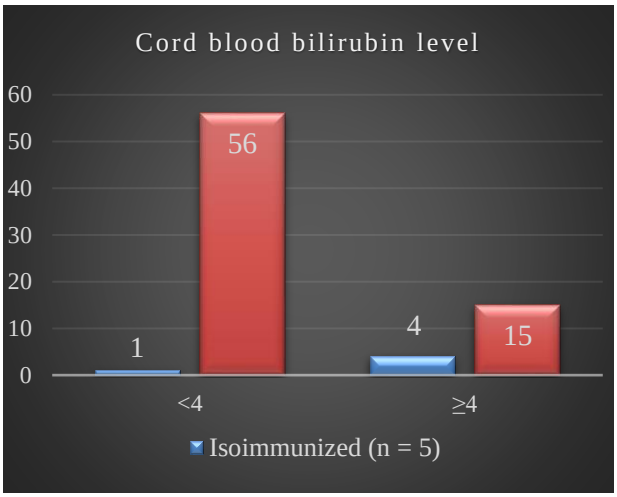

Figure 2: Cord blood bilirubin in isoimmunized and non-immunized pregnancy (n=80).

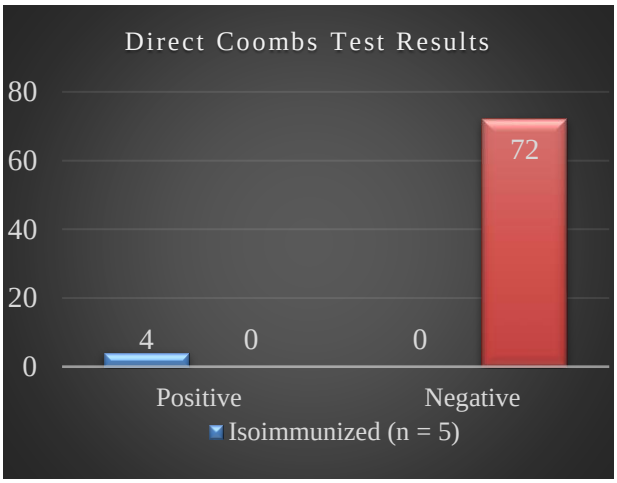

Figure 3: Distribution of newborns according to the Coombs test (n=80).

Figure 1 shows the cord blood haemoglobin levels of newborns. It was markedly lower in neonates born to isoimmunized mothers. Three of these neonates had haemoglobin levels below 12 g/dl-none were observed in the non-isoimmunized group. The majority (75%) had 12-18 g/dl range values. Only non-isoimmunized neonates showed haemoglobin levels exceeding 18 g/dl.

Figure 2 presents that cord blood bilirubin was <4 in 56 cases all cases were non-immunized, and 19 newborns had ≥4 bilirubin. Among them, 4 were isoimmunized, and 15 were of non-immunized mothers. This indicates a strong association between isoimmunization and neonatal hyperbilirubinemia.

Among neonates with available Coombs testing, all four from the immunized group tested positive for the direct Coombs test, confirming immune-mediated hemolysis. In contrast, none of the neonates from immunized mothers were directly Coombs test-positive. The high direct Coombs test positivity rate in immunized cases reflects active antibody-mediated red blood cell destruction. Table 2 presents the neonatal interventions required among the study population. Therapeutic intervention was significantly more common in the immunized group. Four out of five neonates required phototherapy, and three required exchange transfusion. In contrast, most neonates from immunized mothers required no treatment, and none required exchange transfusion. This emphasizes the increased severity of postnatal complications in immunized pregnancies.

Table 3 presents the APGAR score at 5 minutes. A low 5-minute APGAR score (<6) was more frequent in the isoimmunized group (40%) compared to the non-isoimmunized group (12%). This suggests compromised perinatal adaptation in neonates affected by hemolysis, likely secondary to anemia and intrauterine distress.

Table 1: Baseline characteristics of Rh-negative mothers by isoimmunization status (n=80).

Variable	Isoimmunized (n=5)	Non-isoimmunized (n=75)	Total (n=80)
Gravidity ≥3	4	30	34 (42.5%)
Irregular or no ANC follow-up	4	49	53 (66.25%)
Anti-D prophylaxis not taken/inadequate	5	62	67 (83.75%)
History of previously affected fetus	3	0	3 (3.75%)
LSCS (caesarean delivery)	3	59	62 (77.5%)

Table 2: Neonatal interventions required (n=80).

Treatment	Isoimmunized (n=5)	Non-isoimmunized (n=75)	Total
No treatment needed	1	45	46 (57.5%)
Phototherapy	4	6	10 (12.5%)
Exchange transfusion	3	0	3 (3.75%)

Table 3: APGAR score at 5 minutes (n=80).

Score	Isoimmunized (n=5)	Non-isoimmunized (n=75)	Total
<6	2	9	11 (13.75%)
≥6	3	66	69 (86.25%)

DISCUSSION

This study demonstrates a significant impact of Rh isoimmunization on neonatal hematologic parameters and perinatal outcomes. Neonates born to isoimmunized Rh-negative mothers exhibited markedly lower haemoglobin levels, elevated bilirubin concentrations, universal positivity on direct Coombs testing, and higher phototherapy and exchange transfusion rates than neonates born to non-isoimmunized mothers. These findings correspond closely to the classical pathophysiology of hemolytic disease of the fetus and newborn (HDFN), where maternal alloantibodies target fetal red blood cells, leading to hemolysis, anemia, and hyperbilirubinemia.[1] The prevalence of anemia in isoimmunized neonates, with 60% presenting haemoglobin below 12 g/dl, corroborates earlier observations by Moitra et al., who reported significantly higher rates of neonatal anemia in Rh isoimmunized pregnancies.[12] This anemia is primarily due to the destruction of fetal erythrocytes by maternal anti-D IgG antibodies crossing the placenta.

The resulting anemia can cause hypoxia, leading to compensatory mechanisms that may culminate in hydrops fetalis, a severe complication observed in two cases in this study, reflecting the severity of untreated disease.[1,11] Hydrops fetalis, characterized by fetal edema and effusions, is known to be associated with poor perinatal prognosis, often resulting in fetal demise if not treated with intrauterine transfusions or early delivery.[1] Hyperbilirubinemia was observed in 80% of neonates born to isoimmunized mothers, with bilirubin levels ≥4 mg/dl.

This finding is consistent with Khatun and Begum, who documented elevated serum bilirubin levels and increased risk of neonatal jaundice in Rh isoimmunized neonates.[15] Elevated bilirubin results from accelerated breakdown of antibody-coated red blood cells and the neonate's immature hepatic conjugation systems. Untreated hyperbilirubinemia may lead to kernicterus, causing irreversible neurological damage. Kliegman et al. emphasized the critical role of timely phototherapy and exchange transfusion in preventing bilirubin-induced neurotoxicity. These interventions were required in a significant proportion of the isoimmunized neonates in this study.[11]

The direct Coombs test (DCT) was positive in all neonates born to isoimmunized mothers and negative in those born to non-isoimmunized mothers, confirming the immune-mediated mechanism of hemolysis in affected neonates. Eleje et al underscored the importance of DCT as a diagnostic tool in detecting antibody-coated red cells in neonates affected by Rh isoimmunization.[13] A positive DCT is a strong predictor of the need for intensive neonatal management and correlates with the severity of hemolysis.

Treatment modalities such as phototherapy and exchange transfusion were significantly more common among neonates of isoimmunized mothers. Our data show that 80% of these neonates required phototherapy and 60% underwent exchange transfusion, compared to minimal interventions in non-isoimmunized neonates. These findings agree with Nagamuthu et al., who reported higher rates of phototherapy and exchange transfusion in neonates affected by Rh isoimmunization.[14] The need for exchange transfusion reflects the severity of hemolysis and hyperbilirubinemia. It highlights the clinical burden on neonatal intensive care services in regions where prophylaxis and early detection remain suboptimal.

Poor perinatal adaptation, as evidenced by Apgar scores below 6 at five minutes in 40% of isoimmunized neonates compared to 12% in non-isoimmunized neonates, further reflects the consequences of fetal anemia and intrauterine hypoxia. Tripathi and Singh reported similar findings, associating low Apgar scores and increased neonatal morbidity with Rh isoimmunization.[16] These compromised perinatal outcomes necessitate heightened vigilance and immediate neonatal resuscitative care.

Neurological sequelae and perinatal mortality remain significant concerns in Rh isoimmunization. Eleje et al reported high perinatal mortality and long-term neurological morbidity in similar cohorts.[13] The devastating impact of untreated or inadequately treated Rh disease underscores the urgent need for prevention and timely intervention. Maternal factors such as higher gravidity, inadequate antenatal care, and lack of anti-D prophylaxis were notably more prevalent in the isoimmunized group. This aligns with Khatun and Begum, who emphasized the critical role of prophylaxis in preventing maternal sensitization and consequent neonatal complications.[15] Despite the proven efficacy of anti-D immunoglobulin in preventing Rh isoimmunization, gaps in awareness, access, and implementation continue to contribute to the persistence of this preventable condition in many low- and middle-income countries.[14]

The diagnosis and management of fetal anemia before the onset of hydrops fetalis are important in improving perinatal outcomes. Although our setting lacked advanced tools such as middle cerebral artery Doppler ultrasound and the capacity for intrauterine transfusions, Dutta highlights the importance of early screening and close monitoring in at-risk pregnancies to mitigate the risks of severe fetal anemia and hydrops.[1] The absence of these resources in many regions contributes to ongoing morbidity and mortality from Rh disease. This study confirms that Rh isoimmunization exerts a profound negative influence on neonatal hematologic and perinatal outcomes. Prevention through universal and timely administration of anti-D immunoglobulin, early antenatal screening, and strengthening neonatal care infrastructure is essential to reducing the burden of hemolytic disease in Rh-negative pregnancies.

This study is limited by the small sample of isoimmunized mothers, affecting the generalizability of the findings. Being a single-center study, results may not reflect broader population variations. The lack of advanced fetal monitoring tools, like middle cerebral artery Doppler ultrasound, and the absence of intrauterine transfusion facilities limited early detection and management of severe fetal anemia. Additionally, the lack of long-term follow-up data on neonatal outcomes limited the assessment of chronic sequelae related to hemolytic disease.

CONCLUSION

Rh isoimmunization remains a significant cause of neonatal morbidity in Rh-negative pregnancies, shown by anemia, hyperbilirubinemia, and increased treatment needs. This study highlights the need for enhanced antenatal care with universal anti-D prophylaxis and improved fetal monitoring to manage hemolytic disease effectively. Addressing these gaps can reduce adverse perinatal outcomes and improve neonatal survival in resource-limited settings.

Recommendations

To reduce the burden of Rh isoimmunization, universal antenatal screening for Rh-negative status and antibody monitoring should be implemented. Timely anti-D immunoglobulin prophylaxis is essential, especially in resource-limited settings. Strengthening healthcare infrastructure to include advanced fetal surveillance and establishing protocols for intrauterine transfusions would improve outcomes. Increasing awareness among healthcare providers and pregnant women about Rh incompatibility can enhance prophylaxis uptake and interventions. Systematic long-term follow-up of affected

neonates is recommended to manage potential neurodevelopmental complications.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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