Int. J. Pharm. Sci. Rev. Res., ISSN: 0976–044X, 83(1), November – December 2023; Article No. 18, Pages: 112-116; **DOI:** 10.47583/ijpsrr.2023.v83i01.018

Research Article

ABO/Rh Incompatibility in Neonatal Hyperbilirubinemia in a Tertiary Care Teaching Hospital: A Cross-Sectional Study

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Received: 18-09-2023; **Revised:** 23-10-2023; **Accepted:** 30-10-2023; **Published on:** 15-11-2023.

ABSTRACT

Introduction: Maternal-foetal blood group incompatibility can cause haemolytic disease of the foetus and newborn (HDFN), a risk factor for early-onset hyperbilirubinemia and bilirubin-induced neurotoxicity. The present study evaluated the incidence of ABO and Rh incompatibility and its association with neonatal hyperbilirubinemia.

Methods: Observational and cross-sectional study conducted among 257 apparently healthy full-term newborns. Relevant maternal history and birth details were noted. On third day of life, samples of all newborns were sent for laboratory tests (mother's blood group, baby's blood group, unconjugated serum bilirubin and Direct Coomb's test), irrespective of development of clinical jaundice. All children developing neonatal hyperbilirubinemia were treated accordingly.

Results: Most common maternal blood group was A (40.1%) and 86% were Rh positive. Most common newborn blood group was B (37.4%) and 90.3% were Rh positive. 59.1% newborns had either ABO and/or Rh incompatibility. Overall, 50.6%, 5.4% & 3.1% newborns had only ABO, only Rh & both ABO and Rh incompatibility respectively. 30.4% neonates developed hyperbilirubinemia by day 3 and 7.9% were Direct Coombs test positive. ABO-Rh incompatibility was seen in 91% with neonatal hyperbilirubinemia (on day 3) and 45.3% without hyperbilirubinemia. There was a significant association observed between neonatal hyperbilirubinemia and ABO-Rh incompatibility ($p<0.001$).

Conclusion: This study reiterates that babies with Rh incompatibility are more prone to develop serious haemolysis as compared to ABO incompatibility, and demands early identification. Babies with high-risk category can be asked for early follow-up or parents can be counselled for need of delayed discharge if timely follow up cannot be ensured.

Keywords: ABO/Rh incompatibility, Hyperbilirubinemia, Newborn, Haemolysis, Direct Coombs test positive.

INTRODUCTION

Neonatal hyperbilirubinemia is a benign, transient phenomenon occurring in most neonates, [1] resulting from either physiological or pathological processes. [2] Due to an increase in bilirubin synthesis and a decrease in bilirubin clearance, total serum bilirubin (TSB) concentrations rise during the first week of life. [2, 3] Following that, the liver efficiently conjugates and eliminates the bilirubin, and the levels of TSB revert to normal. [1] Because of the potential toxicity of bilirubin, newborns must be monitored to identify those who may develop severe hyperbilirubinemia and, in rare cases, may develop bilirubin encephalopathy or kernicterus, culminating in cerebral palsy and death. [2] Some babies experience severe hyperbilirubinemia during the first week of life, necessitating phototherapy, or even an exchange transfusion. The American Academy of Paediatrics recommends assessing newborns for hyperbilirubinemia prior to nursery discharge by performing a risk assessment and measuring a bilirubin concentration. [4]

Haemolytic disease of the foetus and newborn (HDFN), a risk factor for early-onset hyperbilirubinemia and bilirubin-induced neurotoxicity, can be brought on by maternal-foetal blood group incompatibility. [5] ABO incompatibility and Rhesus (Rh) incompatibility are frequent causes of HDFN. About 25% of pregnancies have foetal-maternal ABO incompatibility, but only 10% of such pregnancies end in haemolytic disease. O group mothers and A or B group foetuses are the most prevalent fetomaternal combinations. Maternal immunoglobulin G (IgG) antibodies traverse the placenta in HDFN cases due to ABO incompatibility and haemolyze foetus and newborn red blood cells, resulting in severe hyperbilirubinemia. [6]

In the Indian population, the prevalence of the Rh-negative blood group is 5%. When a Rh-negative mother is carrying a Rh-positive foetus, the antigen of the foetal RBC may cause an antibody response by the maternal immunologic system because there are no inborn antibodies in the Rhesus blood group system. As the first antibodies to form are of the IgM type, which cannot cross the placenta, these antibodies put subsequent pregnancies at risk for HDFN. The IgG-type anti-D antibodies cross over to the foetus and destroy any foetal red blood cells that are D-positive. [6]

HDFN as a result of ABO incompatibility is clinically milder than that due to Rhesus incompatibility. While the former

is a physiological condition, but may occasionally lead to severe haemolysis in the context of additional aggravating conditions; the latter is preventable and can be avoided, if screened appropriately. Presently, ABO incompatibility is one of the most common causes of neonatal jaundice. [7]

With this background, the present study was conducted with the objectives to determine the incidence of ABO and Rh incompatibility and its association with neonatal hyperbilirubinemia among newborns in a tertiary care teaching hospital of eastern India.

MATERIALS AND METHODS

Study design and study population

The present work was a prospective observational study conducted among 257 apparently healthy full-term newborns. The study was conducted in the Department of Physiology, Biochemistry, Paediatrics and Obstetrics & Gynaecology department of Darbhanga Medical College and Hospital, Laheriasarai, Bihar, India. Cases were obtained from Paediatrics and Obstetrics & Gynaecology department for the period of 6 months from April 2022 to September 2022. Mothers or guardian of babies who did not consent for participation were excluded from the study. Babies with congenital malformations, liver disorders, perinatal asphyxia, known maternal risk factors, pre-term and small-for-gestational age babies were also excluded.

Sample size

In a study by Sahoo et al. [8] the prevalence of hyperbilirubinemia in neonates was 60%. Using these figures along with 95% level of confidence and 10% relative error, the minimum sample size was calculated to be 257.

Ethical approval

The study was initiated after obtaining approval from the Institutional Ethics Committee of DMCH, Laheriasarai and permission from head of the concerned institution.

Study procedure

Mothers of all term, apparently healthy babies meeting the study eligibility criteria were invited to participate in the study. If any new born developed clinical jaundice in first two days of life, blood samples were sent for laboratory tests - mother's blood group, baby's blood group, unconjugated serum bilirubin and Direct Coomb's test (DCT). Relevant maternal history taking and birth details of their newborns were noted.

On third day of life, samples of all newborns were sent for laboratory tests irrespective of development of clinical jaundice. All children developing neonatal hyperbilirubinemia were kept under vigilance and treated with phototherapy or exchange transfusion as indicated. All details were recorded in a case record form.

Data analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 17.0 (IBM Corp., Illinois, Chicago). The mean and standard deviation was calculated for quantitative data. Categorical variables were expressed in proportions. Mann Whitney U test was used to compare mean serum bilirubin levels between those with and without ABO-Rh incompatibility. Chi-square test was used to test for association between neonatal hyperbilirubinemia and ABO-Rh incompatibility. A p-value <0.05 was considered as statistically significant.

RESULTS

Out of 257 neonates included in the study, 142 (55.2%) were males. Majority of the mothers (n=211, 82.1%) were aged between 20-29 years and 61.5% (n=158) were primiparous. The mean (± SD) birth weight was 2.82 (± 0.37) kg; the minimum weight being 2.5 kg and maximum was 4.0 kg. Also, 69.3% (n=178) of the babies were born through normal vaginal delivery.

The most frequently occurring maternal blood group was A, accounting for 40.1% of the mothers, followed by O (26.4%), B (21.4%) and AB (12.1%) was the least common type. 14% of the mothers were Rh negative, while the remaining 86% were Rh positive. In the newborns, the most common blood group was B (37.4%) closely followed by A (34.2%). AB was the least common blood group among the newborns too, comprising of 8.9%. A 90.3% of the babies were RH positive, while the remaining 9.7% were RH negative. (Table 1)

There were 152 newborns who had either ABO and/or RH incompatibility comprising of 59.1% of the study participants. Overall, 50.6% had only ABO incompatibility, 5.4% had only RH incompatibility, while 3.1% had both ABO and RH incompatibility. (Figure 1)

The proportion of neonates who developed hyperbilirubinemia by day 3 was 30.4% (n=78) and 20 babies (7.9%) were found to be positive for Direct Coombs test.

ABO-RH incompatibility was found in 91% of the neonates who had neonatal hyperbilirubinemia on day 3, and 45.3% of those without hyperbilirubinemia. There was a significant association observed between neonatal hyperbilirubinemia and ABO-RH incompatibility (p<0.001). (Table 2)

The mean unconjugated bilirubin in neonates having either ABO and/or RH incompatibility (15.87 (± 3.85) mg/dl) was significantly higher than those born without ABO-RH (11.65 (± 2.41) mg/dl) (p<0.001). The mean unconjugated bilirubin in neonates having RH incompatibility only (19.71 (± 3.24) mg/dl) and both ABO and RH incompatibility (21.97 (± 4.08) mg/dl) were found to be significantly higher as compared to those without incompatibility (p<0.001). The mean unconjugated bilirubin in neonates with ABO incompatibility only (13.10 (± 1.98) mg/dl) was comparable with those without incompatibility (p=0.538). (Table 3)

Out of the 78 babies with neonatal hyperbilirubinemia, 70 babies (89.7%) received phototherapy, and remaining 8 babies (10.3%) underwent exchange transfusion.

Table 1: Distribution of study participants according to maternal and baby blood group and RH status (N=257)

Parameters	Frequency n (%)
Maternal blood group	
A	103 (40.1)
B	55 (21.4)
AB	31 (12.1)
O	68 (26.4)
Maternal RH status	
Positive	221 (86.0)
Negative	36 (14.0)
Baby blood group	
A	88 (34.2)
B	96 (37.4)
AB	23 (8.9)
O	50 (19.5)
Baby RH status	
Positive	232 (90.3)
Negative	25 (9.7)

Figure 1: Pie diagram showing distribution of study participants according to ABO and RH incompatibility (N=257)

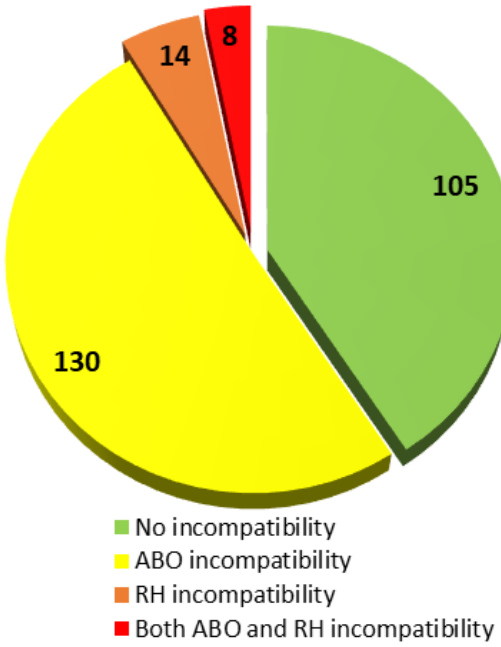

Table 2: Association between neonatal hyperbilirubinemia and ABO-RH incompatibility status (N=257)

ABO-RH incompatibility	Frequency	Neonatal hyperbilirubinemia		p value*
		Present (n=78)	Absent (n=179)	
Yes	152 (59.1)	71 (91.0)	81 (45.3)	**<0.001***
No	105 (40.9)	7 (9.0)	98 (54.7)	

Values are represented as n (%); * p value was calculated using Chi-square test and value <0.05 was considered as statistically significant.

Table 3: Comparison of mean unconjugated bilirubin on day 3 between patients with ABO and RH incompatibility and those without incompatibility (N=257)

ABO and RH incompatibility	Mean (±SD) cord blood unconjugated bilirubin (mg/dl)	p-value
No incompatibility	11.65 (± 2.41)	
ABO incompatibility	13.10 (± 1.98)	0.538
RH incompatibility	19.71 (± 3.24)	**<0.001***
Both ABO and RH incompatibility	21.97 (± 4.08)	**<0.001***
Either ABO and/or RH incompatibility	14.87 (± 3.85)	**<0.001***

DISCUSSION

The present study included 257 apparently healthy, term, AGA newborns, with 55.2% males and the remaining 44.8% were females. All babies included in the study had birth weight >2.5kg with a mean (± SD) birth weight of 2.82 (± 0.37) kg. 61.5% of the mothers were primiparous and 69.3% of the mothers delivered vaginally. Our observations were consistent with that reported by Gupta N et al. [9] where out of 152 neonates, 56.6% were males and 43.4% females, showing a male preponderance. The mean birth weight was comparable between neonates with and without significant hyperbilirubinemia (2.88 ± 0.19 kg vs. 2.88 ± 0.32 kg respectively). There were 55.3% primigravidae and 44.7% multigravida mothers. 40.1% neonates were born through Caesarean section and 59.9% neonates were delivered vaginally. Study by Garg et al. [2] also reported comparable neonatal and maternal characteristics.

Incidence of neonatal hyperbilirubinemia

In our study, hyperbilirubinemia on day 3 was noted in 78 neonates, accounting for 30.4% incidence of neonatal

hyperbilirubinemia. Also, 91% of the babies with neonatal hyperbilirubinemia were found to have ABO and/Rh incompatibility, which was statistically significant (p<0.001). Kardum et al. [10] reported a 14.9% incidence of neonatal hyperbilirubinemia among their newborns in the first 48 hours of life. Garg et al. [2] observed the incidence of neonatal hyperbilirubinemia with first 72 hours of life as 10.8%. Also, Gupta N et al. [8] reported that among their 152 healthy term newborns, significant neonatal hyperbilirubinemia was seen in 11.2% neonates. In another study by Satrya et al. [11] the incidence of hyperbilirubinemia on or before the fifth day of life was 23.9% among their neonates. Study by Jones et al. [12] reported the incidence of clinical jaundice to be as low as 2.1% among their study population of 4069 neonates. The incidence of neonatal hyperbilirubinemia seemed to have a varied range, which may be due to a wider variety of causes of jaundice among the newborns besides haemolytic jaundice, that included physiological jaundice, breastfeeding failure jaundice and breastmilk jaundice etc.

Neonatal hyperbilirubinemia and ABO-Rh incompatibility

There were 152 newborns who had either ABO and/or RH incompatibility comprising of 73.7% of the study participants. Overall, 50.6% had only ABO incompatibility, 5.4% had only RH incompatibility, while 3.1% had both ABO and RH incompatibility. We observed that ABO-RH incompatibility in 91% of the neonates with neonatal hyperbilirubinemia, which was significantly higher than 45.3% of those without hyperbilirubinemia (p<0.001).

Garg et al. [2] reported findings consistent with our observations. Out of 250 newborns in their study, 18.4% cases had ABO incompatibility and 7.2% cases had Rh incompatibility. In neonates with ABO incompatibility, 30.4% cases had significant hyperbilirubinemia, whereas in cases of Rh incompatibility, 11.1% developed significant hyperbilirubinemia. The study found hyperbilirubinemia to be significantly associated with the presence of maternal-foetal blood group incompatibility (p<0.001).

In the present study, 7.9% of the neonates were positive for Direct Coombs test. In the study by Patel AS et al. [13] 90% of the babies with ABO incompatibility developed hyperbilirubinemia within the first 72 hours of life, among them majority had physiological jaundice. Also, in ABO incompatibility, DCT was positive in only 9% cases and in Rh incompatibility group it was positive in 25%, reiterating that in Rh incompatibility, there is severe iso-immunization.

In our study, the mean unconjugated bilirubin in neonates having RH incompatibility only and both ABO and RH incompatibility were found to be significantly higher as compared to those without incompatibility (p<0.001); while the mean value for neonates with ABO incompatibility was comparable with those without incompatibility (p=0.538). This result indicates that, among our study participants, jaundice occurring in neonates with Rh incompatibility in isolation or along with ABO incompatibility was more severe than the mean values for neonates developing jaundice due to ABO incompatibility alone. Cumulative evidence in literature point that, unlike HDFN arising due to Rh incompatibility, that due to ABO incompatibility is asymptomatic or produces mild hyperbilirubinemia and anaemia. [13,14] ABO HDFN is seen in some blood group A or B infants born to group O mothers who produce IgG anti-A and anti-B, which are capable of crossing the placenta. The disease is milder than Rh HDFN likely because A and B antigens are poorly developed on foetal and newborn RBCs, and other cells and tissues express A and B antigens, which reduces the amount of maternal antibody directed against foetal RBCs. [14] The DAT result for the newborn with ABO HDFN is only weakly positive and may be negative. [14] In most cases of ABO HDFN, neonatal hyperbilirubinemia remains within physiological limit. It is easily reversible, with minimal morbidity, and without any mortality. [13]

CONCLUSION

Neonatal hyperbilirubinemia has been found to be the most frequent causes for readmission of the newborns. Even with the most careful and efficient prenatal screening techniques, it is least likely to anticipate or foretell which couple will have a foetus that will suffer ABO incompatibility hyperbilirubinemia. Additionally, the immunological responses arising out of incompatibility cannot be averted. Therefore, hyperbilirubinemia resulting from ABO incompatibility must be actively treated whenever indicated. However, in cases of Rh incompatibility, routinely administering mother with anti-D can effectively prevent serious haemolysis in subsequent pregnancies. The present study reiterates that babies with Rh incompatibility are more prone to develop serious haemolysis as compared to ABO incompatibility, and demands early identification. Parents who have infants in the high-risk category may be counselled about the necessity for a delayed discharge or requested for an early follow-up.

Acknowledgement: We are thankful to the healthcare workers and faculty members of Department of Biochemistry, Paediatrics and Obstetrics & Gynaecological of Darbhanga Medical College and Hospital, Laheriasarai, Bihar for their support.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.