

A Comparative Study of Cord Blood Bilirubin Levels and Duration of ICU Stay in Maternal ABO and Rh-D Antibody Mediated Haemolytic Disease of Newborn

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ABSTRACT

BACKGROUND

Lifespan of fetal or neonatal red cells is decreased by placental transfer of maternally produced IgG antibodies in immune mediated Haemolytic Disease of Fetus and Newborn (HDFN). We wanted to compare cord blood bilirubin levels and the duration of ICU stay in maternal ABO and RH-D antibody mediated haemolytic disease of fetus and newborn.

METHODS

This research work was designed as a cross sectional study. 154 neonates who were diagnosed to have HDFN were studied in the Department of Transfusion Medicine and Pediatrics, Government Medical College, Trivandrum, Kerala. 5 mL umbilical cord blood was collected at the time of delivery. The collected sample was used for analyzing bilirubin levels. Duration of ICU stay of the neonates was recorded. SPSS version 16 was used for statistical analysis.

RESULTS

The mean cord blood bilirubin levels in ABO haemolytic disease was 5.16 ± 1.91 . The mean cord blood bilirubin level in Rh-D haemolytic disease was 4.15 ± 1.10 . In infants with ABO haemolytic disease, 55 (50 %) stayed in ICU for 5 days. Remaining 50 % stayed in ICU ranging from 6 - 14 days. Among infants with Rh-D haemolytic disease 22 (50 %) stayed in ICU for 5 days. Remaining 50 % had stayed in ICU for 6 - 15 days. Mean duration of stay of infants with ABO haemolytic disease in ICU was 7.2 ± 2.9 days. The mean duration of stay of infants with Rh-D haemolytic disease in ICU was 6.8 ± 2.7 days. Mean values of cord blood bilirubin levels in both categories of haemolytic disease (ABO and Rh-D HDFN) were compared using independent t test. Significant difference was observed with a p value of 0.001. Mean values of duration of stay of neonates in both categories of haemolytic disease (ABO and Rh-D HDFN) in ICU were compared using independent t test. No significant difference was observed during the analysis.

CONCLUSIONS

Umbilical cord blood bilirubin levels were significantly higher in ABO haemolytic disease as compared to Rh-D haemolytic disease. Even though duration of stay in ICU was higher for neonates suffering from ABO HDFN as compared to Rh-D HDFN category, the difference was not statistically significant.

KEYWORDS

Haemolytic Disease of Fetus and Newborn, Umbilical Cord Bilirubin, Hyperbilirubinemia, ICU Stay, ABO HDFN, Rh-D HDFN

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BACKGROUND

In haemolytic disease of newborn, lifespan of red blood cells of fetus or neonates is significantly decreased due to maternal IgG antibodies which are placentally transferred during gestation.¹ This immune mediated haemolytic disease can be caused by different types of red cell antibodies. Most common types of antibodies involved in haemolytic disease are anti-A, anti-B and anti-D. Immunization to D antigen occurs when maternal circulation is exposed to fetal red cells through placental transfer or by transfusions.²

The disease is accelerated by the hypoxia induced by anaemia and toxic effects resulted from increased levels of bilirubin.¹ The liver of neonates is immature as compared to adult ones. Due to this reason, soon after birth, plasma levels of unconjugated bilirubin will suddenly get elevated. This may cause central nervous system damage to infant's brain.³

The 'Kerala Model' of development in public health sector had its improvements in almost all areas of living which includes lower levels of infant mortality, comparatively low growth of population, higher levels of literacy rates, increased life expectancy, rendering high quality health services etc.⁴ Hence evaluating the pattern of disease by comparing HDFN caused by ABO and Rh-D antibodies was planned to be utilized as a tool for development of novel management protocols for this preventable factor of fetal loss.

METHODS

The study was designed as a comparative cross sectional one. 154 neonates who were having HDFN was included in this study. The subjects for this research were selected by random sampling method. Study was analysed and approved by Human Ethical Committee and review board of concerned hospital. (IEC no; 02 / 48 / 2012 / MCT / 16 - 02 - 2012) Study was done for a period of 18 months in 2012. Setting for this research was Department of Transfusion Medicine and Paediatrics, Government Medical College, Trivandrum.

The neonates having increased bilirubin levels who fulfilled the inclusion criteria were included in this study. Parents of all neonates were counselled, and consent was obtained. Inclusion criteria included for ABO haemolytic disease included hyperbilirubinaemia on day 1, materno-neonatal ABO incompatibility, ABO maternal IgG titre ≥ 32 and neonatal DAT (Direct Antiglobulin Test) and / or elution positive or presence of maternal antibodies in cord blood serum. For Rh-D haemolytic disease the inclusion criteria were maternal antibody screen positivity for anti-D, absence of materno-neonatal ABO incompatibility, positive direct antiglobulin test and elution in neonate along with hyperbilirubinaemia on day 1.

Exclusion criteria included other causes for hyperbilirubinaemia like twin-to-twin transfusion, infection or sepsis, Dubin-Johnson syndrome, hereditary spherocytosis, G6PD deficiency, Thalassemia, biliary atresia,

cholestasis, cephalohaematoma, hypothyroidism, cystic fibrosis etc.

Demographic details were noted. 5 mL umbilical cord blood was collected to assess bilirubin levels. Duration of stay in ICU was recorded.

Analysis of statistical data was performed using SPSS software version 16. Mean values and standard deviations were calculated for continuous variables in the study. Qualitative data of neonates was expressed as frequencies along with percentages. Independent t test was used for comparison of mean values between groups. P value < 0.05 was considered as statistically significant.

RESULTS

Among 154 cases of haemolytic diseased infants, 71.4 % (110) was due to ABO antibodies and 28.6 % (44) was due to Rh-D antibodies.

Parameter	Frequency	Percentage
Hyperbilirubinaemia due to ABO HDFN	110	71.4
Hyperbilirubinaemia due to Rh-D HDFN	44	28.6
Total Hyperbilirubinaemia cases	154	100

Table 1. Distribution of ABO and Rh-D HDFN

Parameter	ABO HDFN (n = 110)	Rh-D HDFN (n = 44)	P Value
Mean (mg %)	5.16 $\pm$ 1.91	4.15 $\pm$ 1.10	0.0012*

Table 2. Comparative Analysis of Cord Blood Bilirubin Values

* indicates statistically significant difference at P < 0.05

The mean cord blood bilirubin levels in ABO haemolytic disease was 5.16 ± 1.91 . While the mean cord blood bilirubin levels Rh-D haemolytic disease infants was 4.15 ± 1.10 . The median cord blood bilirubin levels in ABO and Rh-D haemolytic disease was 4.21 and 4.15 mg % respectively. Minimum bilirubin levels in ABO haemolytic disease was 3.01 mg %. Minimum level in Rh-D haemolytic disease was 1.79 mg %. Maximum bilirubin levels in ABO and Rh-D haemolytic disease was 10.659 and 8.81 mg % respectively.

Mean values of cord blood bilirubin in neonates with ABO and Rh-D haemolytic disease were statistically compared using independent t test. Significant difference was observed with a p value of 0.001. 95 % Confidence interval was 0.40 to 1.61 and t value was 3.29.

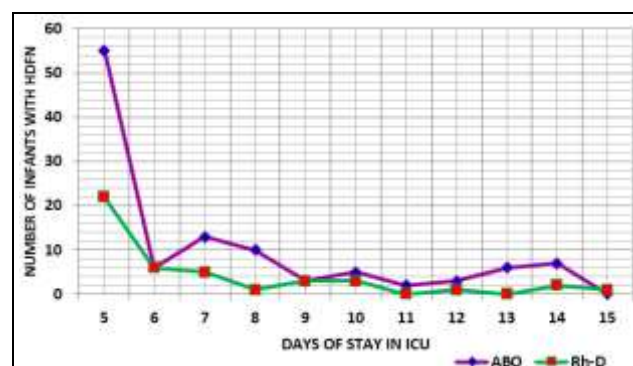


Figure 1. Duration of Stay of Infants with ABO and Rh-D HDFN in ICU

In infants with ABO haemolytic disease 55 (50 %) stayed in ICU for 5 days. Remaining 50 % stayed in ICU ranging from 6 - 14 days. Among infants with Rh-D haemolytic disease 22 (50 %) stayed in ICU for 5 days. Remaining 50 % had stayed in ICU from 5 - 15 days.

Parameter	ABO HDFN (n = 110)	Rh-D HDFN (n = 44)	P Value
Mean duration of stay in days	7.2 ± 2.9	6.8 ± 2.7	0.4770

Table 3. Comparative Analysis of Stay of Infants with HDFN in ICU

Mean duration of stay of infants suffering from ABO haemolytic disease in ICU was 7.2 ± 2.9 days. While mean duration of stay of neonates having Rh-D HDFN, in ICU, was 6.8 ± 2.7 days. Median duration of ICU stay of infants with both ABO and Rh-D haemolytic disease were same, 5.5 days. All neonates with haemolytic disease were observed in intensive care rooms for a minimum of 5 days. A maximum of ICU stay was 14 days and 15 days for ABO and Rh-D HDFN respectively. Mean values of duration of stay of neonates with ABO and Rh-D HDFN in ICU were compared using independent t test. Mean difference was 0.37. No significant difference was observed with a p value of 0.477.

DISCUSSION

Dinesh et al., found that the ABO incompatibility between mother and baby was one of the important causes of jaundice among neonates in areas having high human development index.⁵ According to Schonitzer et al., nearly 5 % of neonatal hyperbilirubinaemia which needed therapy was due to ABO HDFN.⁶ Karel Polacek found out that among Rh-D HDFN affected neonates, bilirubin levels were very frequently increased in cord blood. He added that it was quite an essential investigation which can be used in deciding the modality of treatment.⁷ Both ABO and Rh-D HDFN necessitates different patterns of treatment and stay in intensive care centers. This study has tried to compare cord blood bilirubin levels and duration of stay in ICU among neonates affected by ABO and Rh-D HDFN.

The mean cord blood bilirubin levels in ABO and Rh-D HDFN among infants admitted in neonatal intensive care centres were 5.16 ± 1.91 and 4.14 ± 1.1 respectively. These mean values of cord blood bilirubin were compared using independent t test. Significant difference was observed and the p value was 0.0012.

The bilirubin levels among neonates having both ABO and Rh-D HDFN were on higher side, as compared to normally delivered neonate's reference values.⁷ Gilja et al., found out that smaller levels of red cell destruction frequently raised neonatal bilirubin levels among ABO incompatible infants.⁸ In a study by Valentine et al., 1 in 70 - 180 ABO incompatible infant developed jaundice with increased bilirubin levels within 24 hours of birth.⁹ Few ethnic groups may express strong, numerous and branched A or B antigen sites in red blood cell surface. Sherer et al., concluded that among such neonates, bilirubin levels were

higher, and anaemia was severe as compared to other ABO incompatible infants.¹⁰ He also opined that more nucleated red cells were found in peripheral smear of such infants. He added that infants had even had hydrops fetalis.¹⁰ ABO antibodies are naturally occurring and if it is IgG type, it can transfer through placenta. Thus, first ABO incompatible infant was at risk for significant haemolysis followed by hyperbilirubinemia and anaemia.¹¹ In a study by Rosenfield and Ohno, bilirubin levels was only higher in infants born to ABO incompatible mothers. But when they analysed the bilirubin levels of ABO incompatible infants with DAT positivity with ABO incompatible infants with DAT negativity, the values were significantly higher among positive antiglobulin test group.¹²

Bowman et al., found out that during birth, nearly 50 % of neonates affected with Rh-D HDFN had no dangerous increase in bilirubin levels. Even though they were DAT positive, they required no treatment.⁷ Harvey Klein and David Anstee opined that in Rh-D haemolytic disease, neonates might have a strong DAT positivity, still having no clinical signs of significant disease.¹³ In par with above mentioned findings, Mollison and Cutbush found out that cord bilirubin levels in neonates suffering from haemolytic disease was not strongly correlated with disease severity.¹⁴ Crawford et al., observed only a minor increase in osmotic fragility in haemolytic disease. They also found spherocytosis in severe cases of Rh-D haemolytic disease of newborn.¹⁵ In this study the bilirubin levels among ABO HDFN study group was significantly higher as compared to Rh-D HDFN study group. Hughes Jones et al., found out that anti-D levels in cord blood were not correlating with cord bilirubin concentration.¹⁶ This result was also not supporting our study results. Bowman et al., observed that 25 % of Rh-D affected infants were extremely jaundiced. They also found out that few neonates with very high bilirubin levels developed kernicterus or even died in disease course.⁷ This result was in par with our study findings.

Mean duration of stay of infants with ABO haemolytic disease in ICU was 7.2 ± 2.9 days. The mean duration of stay of Rh-D HDFN neonates was 6.8 ± 2.7 days. Mean values of duration of stay of neonates with ABO and Rh-D HDFN in ICU were compared using independent t test. No significant difference was observed among study groups. There are various modalities of treatment for HDFN which includes phototherapy, IVIg (Intra-Venous Immuno-Globulin) injections and exchange transfusion.¹³ Most of these treatment modalities are performed in ICU. Thus duration of stay in ICU is always related to modality of treatment.

Vreman et al., concluded that moderate or severe hyperbilirubinaemia in full-term infants with ABO haemolytic disease can be managed successfully by modern phototherapy.^{17,5} Likewise Osborn et al., opined that phototherapy as a treatment modality had usually prevented further rise in serum bilirubin levels. Thus, it has been found beneficial in decreasing the number of days of ICU stay.¹⁸ Murray concluded that in ABO haemolytic disease, high dose IVIg and exchange transfusion will be beneficial for neonates whom express strong A and B antigens in red blood cells. Allen et al., observed that in Rh D haemolytic

disease, exchange transfusion increased the survival rate of neonates. That further helped in decreasing the risk of kernicterus.¹⁹ Harvey Klein and David Anstee pointed out that in severe categories of HDFN, intrauterine transfusions has decreased the rate of exchange transfusion drastically.²⁰ Sproul et al., noticed that the exchange transfusion removed one-fourth of circulating bilirubin content and majority of antibody coated red blood cells. However, after exchange transfusion, in many a neonate, a rapid rebound of serum bilirubin level was common, and it necessitated additional exchange transfusions.²¹ Thus exchange transfusion in Rh-D HDFN usually extended stay of neonates in ICU. However even if the above mentioned authors opined that the rate of exchange transfusion is higher in Rh-D HDFN as compared to ABO-HDFN, there was no significant increase in duration of ICU stay in former category. Eugene Kaplan opined that in severe ABO haemolytic disease, phototherapy can be solitary modality of treatment, however it does not exclude exchange transfusion.²² This might be a reason for above mentioned finding in this study.

While analysing hyperbilirubinemia in neonates, it is important to consider other aetiological factors which can result in same.²³ Hyperbilirubinemia in neonates can be broadly classified as physiological jaundice, pathological jaundice, jaundice due to breastfeeding or breast milk and haemolytic jaundice.²⁴

Physiological jaundice is the most common type with no much serious consequences.²³ Jaundice usually appears between 24 - 72 hours after birth and between peaks by 4 - 5 days in term neonates and 7th day in preterm, and disappears by 10 - 14 days.²⁵ Bilirubin levels which are increased as compared to age adjusted normal range and those requiring intervention is described as pathological jaundice. Appearance of jaundice in first day of life, those values going beyond 5 mg / dl / day, existence of clinical jaundice more than 2 weeks and conjugated bilirubin is categorized under this type of jaundice.²³

Jaundice in breast fed babies usually appears between 1 - 3 days after birth. The bilirubin value peaks by 5 - 15 days and usually disappears by 20 - 24 days.²⁶ A diagnosis of breast milk jaundice should be investigated especially if it is mainly unconjugated bilirubin, other causes of jaundice have been ruled out, the neonate is having good health and gaining weight adequately.²⁷

The most common causes of haemolytic jaundice include Rh-HDFN, ABO incompatibility, HDFN due to minor blood group system antibodies and G-6-PD deficiency. Investigations for G6PD deficiency should be performed in infants with severe jaundice with a family history of significant jaundice or in a geographic origin associated with G-6-PD deficiency.²⁸ Unconjugated bilirubin levels are raised in both breast milk jaundice and jaundice due to haemolytic anaemias. Haemolytic causes for hyperbilirubinemia, must be considered and worked up. Work up should include direct Coombs' testing, checking blood counts and peripheral blood smear evaluation.²⁹

The difference of other jaundice due to HDFN and other categories is that, when managed promptly, the former has good prognosis. Carolien Zwiers found out that with the introduction of intravascular IUTs and implementation of

noninvasive diagnostics for fetal anaemia, survival of neonates have increased markedly.³⁰ Several drugs that are being tried as alternative treatment options for hyperbilirubinemia, includes, albumin, phenobarbitone, zinc, clofibrate, erythropoietin, vitamins like B - 12 and E etc.. However long-term follow-up studies with larger sample size and multi centric analyses are needed for such modalities.³¹

Thus this study it is clearly portraying the fact that serum bilirubin levels in is significantly high in neonates with Rh-D HDFN as compared to ABO HDFN. But it doesn't mean that the duration of stay in ICU is higher for former group. So the protocols which are to be implemented for treatment of HDFN may establish duration of ICU stay same for any class of HDFN. However, a stringent observation and care should be there for Rh-D HDFN neonates who get admitted to neonatal intensive care centres.

CONCLUSIONS

Umbilical cord blood bilirubin levels were significantly higher in ABO-D HDFN as compared to Rh-D HDFN. Even though duration of stay in ICU was higher for neonates suffering from ABO HDFN as compared to Rh-D HDFN, the difference was not significant.

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