Original Article

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Neonatal Outcome of Rh-D Alloimmunisation in Antenatal Women Attending a Tertiary Care Hospital

Paediatrics Section

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ABSTRACT

Introduction: Haemolytic disease of foetus and newborn due to Rh-D alloimmunisation was one of the grave complications of pregnancy years back, which contributed for a number of perinatal deaths and disabilities. Yet, advancement in technologies used for early detection and treatment of haemolytic disease of newborn as well as better neonatal care has further contributed to bring down the magnitude. Our study attempts to follow-up Rh-D alloimmunised pregnancies and collect data regarding the perinatal and postnatal plight of affected neonates.

Aim: To follow-up Rh-D alloimmunised pregnancies and describe the perinatal and postnatal characteristics of affected neonates.

Materials and Methods: This two year prospective study was done on Rh-D alloimmmunised women. Diagnosis of haemolytic disease of newborn due to Rh-D alloimmunisation was confirmed if a positive Direct Coomb's Test (DCT) was found in an Rh-D positive baby. Severity of disease in terms of haemoglobin and bilirubin levels and presence of hydrops were assessed. Newborns were followed-up till discharge.

Results: Out of 2496 Rh-D alloimmunised women, 78 antenatal cases were found positive for anti D-antibodies. Frequency of haemolytic disease of foetus and newborn was 57 out of 64 cases which were followed, four newborns were DCT negative although, Rh-D positive and three newborns were Rh-D negative. Mean cord Hb in unaffected newborns were significantly higher. Twenty nine out of 58 live born newborns needed no treatment. Four cases (6.25%) needed exchange transfusion. Out of 60 live born infants, 59 survived. Overall survival rate of newborns in 64 alloimmunised pregnancies was 92.18%. The survival rate in live borns was 98.33%.

Conclusion: Severe cases of haemolytic disease of newborn in Rh-D alloimmunisation is limited to <10 % and after the advent of better neonatal care and monitoring services survival rates of affected newborns are much higher.

Keywords: Exchange transfusion, Haemolytic disease, Jaundice, Newborn

INTRODUCTION

Haemolytic disease of foetus and newborn due to Rh-D alloimmunisation was one of the grave complications of pregnancy years back, which contributed for a number of perinatal deaths and disabilities. After introduction of postpartum and additional antepartum prophylactic Rh-Ig, the frequency of Rh-D alloimmunisation could be sustained well below 0.2% [1]. Advancement in technologies used for early detection and treatment of haemolytic disease of foetus and newborn as well as better neonatal care has further contributed to bring down the magnitude of severe Haemolytic Disease of Newborn (HDN). The adequacy of neonatal care facilities which reflects on perinatal morbidity and survival rate is a crucial factor in deciding the plight of affected cases. Our study attempts to follow-up Rh-D alloimmunised pregnancies and collect data regarding the perinatal and postnatal plight of affected neonates. This can be useful in devising programmes to reduce the morbidities of this preventable cause of foetal loss.

In the absence of cordocentesis and foetal sampling, cord blood haemoglobin, bilirubin and DCT are the earliest laboratory parameters available for evaluation of disease status and often used for grading of disease severity [2]. Correlation between these parameters and need for intensive treatment is also examined in this study.

In the 1950s, before the development of exchange transfusion, amniocentesis, intrauterine transfusion and early induction of labour, mortality from stillbirths and neonatal deaths was about 15 per 1000 births, equivalent to well over 1000 deaths a year [1]. After the introduction of the above technical improvements there was a gradual reduction in mortality [3]. Keeping in view the limited resources and non availability of treatments

like intrauterine transfusion in our institution we attempted to examine and compare the outcome of Rh-D alloimmunisation in terms of disease severity, treatment needed and mortality. Thus, this study was conducted to describe the outcome of Rh-D alloimmunisation in neonates in terms of disease severity, treatment needed and mortality.

MATERIALS AND METHODS

This two year prospective study was done on 64 Rh-D negative antenatal cases whose blood samples were found positive for anti Rh-D antibodies by Indirect Anti-human globulin Test (IAT) at Government Medical College, Trivandrum, Kerala, India between the period of October 2008 to September 2010, in collaboration with Department of Transfusion Medicine and Paediatrics.

Antenatal women found to be positive for anti-D IgG antibody by indirect anti-human globulin test with pooled O positive red cells were included in the study. While the subjects who were not willing to participate in the study, co-existing ABO incompatibility and being not amenable to follow-up were excluded from the study.

Sample size was calculated as 53 using the formula for cross sectional designs i.e., $n=Z^2 P(1–P)/d^2$

Where: Z =1.98 (95% confidence interval); P=65% expected prevalence of DCT positivity among Rh-D sensitised antenatal women, and precision; (d) chosen was 13 (20% of P).

To allow for the cases that may be excluded or may have missing data we studied a slightly higher number i.e., 64. All sensitised women included in the study, were counselled separately about the nature and effects of the study. A written consent was obtained from the patients who were finally included in the study. The study was approved by the Institutional Research Committee. IAT was done on blood samples of Rh-D negative cases during the study period and IAT positive cases were further evaluated. Fresh 10 mL samples were collected from 78 IAT positive cases. Cell and serum were separated. ABO grouping (forward and reverse) and Rh-D typing were done by tube method. Rh-D negative status is confirmed by weak D test. Samples were screened for the presence of anti D antibodies by Indirect Coomb's test by tube method and confirmed by LISS-COOMBS gel cards. History was elicited as per proforma. Antibody titration was done on IAT +ve samples by tube method. Titre was recorded and serum preserved frozen at -20^{0}C deep freezer for future reference. Antibody was identified as anti D in frozen samples at a later period using 11 cell panel in order to exclude the cases with presence of non-D antibodies. Patients were followed-up monthly till 28 weeks and thereafter biweekly. Serial antibody titres were obtained. Method of termination of pregnancy, any intervention and gestational age at delivery were observed. Immediately after delivery, cord blood samples were tested for ABO blood group (forward) and Rh typing. Rh-D negative status was confirmed by weak D testing, Cord blood haemoglobin, DCT, cord blood bilirubin and peripheral smear of newborn also were tested. The incidence of DCT positivity and haemolytic disease were noted. According to institutional policy all babies born to mothers with antenatal antibody screen positive were immediately shifted to NICU. Diagnosis of haemolytic disease of newborn due to Rh-D alloimmunisation was confirmed if a positive DCT was found in an Rh-D positive baby. Evidence of erythrocyte destruction like raised reticulocyte count, haemolytic picture in peripheral smear along with Increase in bilirubin and decrease in haemoglobin in cord blood and subsequent samples were also diagnostic. For diagnosis and treatment of hyperbilirubinaemia chart by American Academy of Paediatricians [4] was followed. Baby was followed-up till discharge. Haemoglobin, bilirubin and reticulocyte count was done at 14 days in review clinic. Need for treatment namely phototherapy, IVIg, exchange transfusion, number of blood transfusions needed and components given also noted. The severity of disease was graded as mild, moderate and severe according to grading proposed by Bowman SM et al., [5] Hydrops and stillbirths included in severe cases. Cases with cord blood haemoglobin >12 gm% and bilirubin <3.5 gm% included in mild. Moderate disease was classified as cord blood haemoglobin <12 gm% and bilirubin >3.5 gm%. Intensity of treatment was graded as follows: 0-No treatment;1-Phototherapy alone; 2-Photo and IVIG; 3-Exchange transfusion; 4-Multiple exchange transfusions [6].

STATISTICAL ANALYSIS

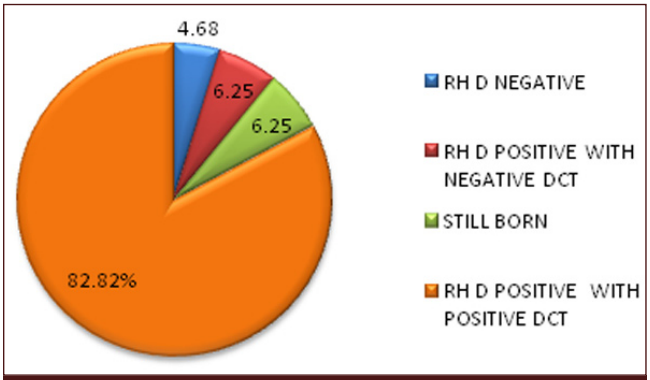
Statistical data were analysed by using SPSS software version 16.0. Continuous variables were expressed as mean ± standard deviation and qualitative data was expressed as percentage. Independent-'t'-test was used for comparing quantitative data between groups. Categorical variables were compared using chi square test. All p-values were two tailed and values of $p<0.05$ was considered statistically significant. Correlations between variables were done using Pearson's correlation test.

RESULTS

Total number of Rh-D negative women in whom IAT for anti D antibody was done in Deparment of Transfusion Medicine during study period were 2496. Out of these, 78 antenatal cases were found positive for anti D antibodies (3.1%). After excluding seven cases of coexisting ABO incompatibility and seven cases who lost follow up, 64 cases were included in the study.

Frequency of haemolytic disease of foetus and newborn was 57 out of 64 cases. Four newborns were DCT negative although Rh-D positive and three Newborns were Rh-D negative.

Newborns who were Rh-D positive and DCT positive, born to Rh-D alloimmunised mothers, were considered as affected with haemolytic disease. Stillborn with clinical evidence of hydrops were also taken as affected [Table/Fig-1]. Perinatal characteristics of neonates are described in [Table/Fig-2]. Mean values of cord blood haemoglobin, cord blood bilirubin and birth weight in affected and unaffected newborns were compared using Independent sample 't' test [Table/Fig-3].



[Table/Fig-1]: Blood group and DCT status of Neonates born to Rh-D alloimmunised mothers.

Variables	Characteristic Features
Mode of Delivery	Normal vaginal: 19 (29.7%)
	Induced vaginal:21 (32.8%)
	LSCS:24 (37.5%)
Foetal Maturity at Delivery	Term-40 (62.3%)
	Preterm-20 (31.5%)
	IUD-4 (6.2%)
Growth Status at Delivery	Small for Gestational Age-24 (37.5%)
	Adequate for Gestational Age-36 (56.3%)
	IUD-4 (6.2%)
Gender	Male-38 (59.4%)
	Female-26 (40.6%)
DCT Grade	Negative-4
	Grade 1-14
	Grade 2-29
	Grade 3-3
	Grade 4-8
Survival at the end of 1 month	59/60 live borns (98.3%)

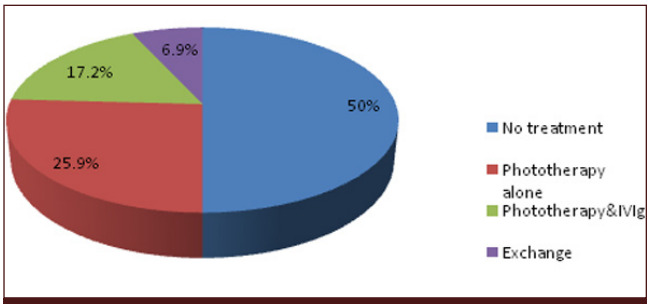
[Table/Fig-2]: Summary of perinatal characteristics of neonates born to Rh-D sensitised mothers.

Category	Number	Mean cord blood Haemoglobin	t value	Mean cord blood Bilirubin	t value
Affected	53	13.77±2.38	3.8 (p<0.001)	2.74±.973	2.427 (p=.018)
Unaffected	7	17.77±0.5		1.84±.053	

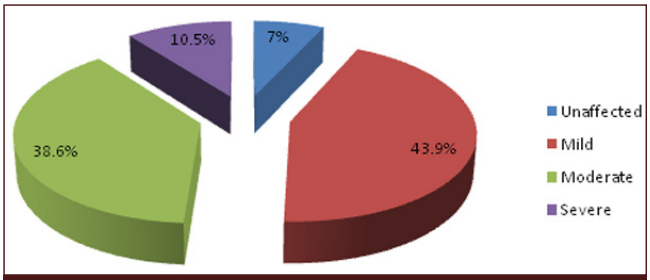
[Table/Fig-3]: Comparison between mean cord blood haemoglobin and bilirubin values in affected and unaffected newborns.

Mean cord Hb in unaffected newborns were significantly higher. Significant increase in cord bilirubin values were observed in affected newborns when compared with unaffected. No significant difference in birth weight was observed between affected and unaffected newborns. Twenty nine out of 58 live born newborns needed no treatment. Various treatments followed are depicted in [Table/Fig-4].

[Table/Fig-5] shows the categorisation of cases according to severity. Red blood cell transfusion was required in fifteen cases. Out of these 15, four cases needed multiple top up transfusions. Three newborns needed platelet concentrates and only one required fresh frozen plasma. Out of 54 affected (DCT positive) newborns only 4 (7.5%) needed exchange transfusion. Overall incidence of exchange transfusion in 64 cases studied were 6.25%. Of 48 infants whose haemoglobin levels were examined in review clinic, 2 had anaemia and minimal jaundice requiring readmission. Total 46 infants had normal haemoglobin and bilirubin levels. Remaining 12 cases did not attend the review clinic at two weeks.



[Table/Fig-4]: Treatment options adopted for Neonates born to Rh-D alloimmunised mothers.



[Table/Fig-5]: Severity of the disease in Rh-D positive newborns of alloimmunised mothers.

In all 59 of 60 live born infants survived. Overall survival rate of newborns in 64 alloimmunised pregnancies was 92.18%. The survival rate in live borns was 98.36%.

Cord haemoglobin values showed a significant negative correlation (r-0.264, p=0.048) and cord bilirubin showed a significant positive correlation (r=0.394, p=0.004) with

increased intensity of treatment. Intensity of treatment varied from no treatment to exchange transfusion. DCT grade had no correlation with intensity of treatment.

Only cord blood haemoglobin was found to have a correlation with number of red cell transfusions needed in a newborn (r-0.422, p=0.001) with decreasing cord blood haemoglobin values, number of RBC transfusions needed in hospital admission period was seen to be increasing. Cord blood bilirubin and grade of DCT did not have a significant correlation with number of RBC units transfused.

DISCUSSION

The frequency of affected children found in our study was 57 out of 64 alloimmunised cases. Many authors have reported that more than half of the newborns of alloimmunised mothers are either unaffected or mildly affected [5,7,8]. In our study 7% were unaffected and 43.9% were mildly diseased together constituting a 50% and severe cases going into hydrops were 10%. Intrauterine transfusion was not given to any.

A study done in 1960s describe the trend of exchange transfusions showed a fivefold increase in exchange transfusions for treatment of HDN in a span of 10 years [9]. A more recent study reported seven exchange transfusions out of 26 affected cases. Cynthia SA et al., reported 300 newborn infants with Rh haemolytic jaundice and a total of 143 patients underwent 207 exchange transfusions [10]. The rate of increase in the serum bilirubin levels (>0.5 mg/dL/hour) was the main indication for exchange transfusion here.

With the advent of specific guidelines and advance options like IVIg the incidence of exchange transfusion was found to be decreasing [11]. In our study majority of cases required no treatment, exchange transfusion was done in much lesser number of newborns 4/64 (6.25%) and phototherapy was the main mode of treatment with additional IvIg in some cases.

Researchers have attempted to estimate the proportion of Rh-D positive offsprings in an Rh-D negative mother [12]. Results close to 90% of neonates being Rh-D positive are observed by Chattopadhyaya D et al., when mother is RhD negative and biological father is RhD positive [13]. We observed Rh-D negative blood group in 5% offsprings of alloimmunised mothers and the rest were Rh-D positive but it is to be noted that only ICT positive antenatal women were included.

Mollison PL et al., have shown the close relationship between the haemoglobin concentration in cord blood and the infant's chance of survival long back [14]. Davidson LT et al., found the relationship between the concentration of foetal bilirubin and the subsequent intensity of bilirubinaemia of the newborn [15]. The higher the concentration of bilirubin in the cord blood, the higher usually is the maximum of the serum bilirubin curve within the first few days after birth [16].

Prognostic value of the haemoglobin concentration versus the combined estimation of the haemoglobin and bilirubin concentration in the cord blood as the most reliable guide in the treatment of haemolytic disease of the newborn was explored by some authors [17,18]. On comparing cord haemoglobin and bilirubin and haemoglobin with intensity of treatment we found a negative correlation with cord haemoglobin and a positive correlation with cord bilirubin. Newborns with higher cord haemoglobin and lower cord bilirubin needed no treatment or only phototherapy. However, neither bilirubin nor cord DCT correlated with number of transfusions received by an affected newborn. Cord haemoglobin showed significant negative correlation with number of RBC transfusions. DCT in offsprings of Rh-D alloimmunised mothers were found positive in 82.82% infants of alloimmunised mothers in our study. A study on 18800 pregnant women sensitised with various antibodies, 160 cases were due to anti D antibodies and 41 offsprings affected with HDN [19]. Cord DCT grading did not correlate with intensity of treatment in our study. Survival above 92% was reported in our study similar to Craig S et al., [20].

LIMITATION

Study is a hospital based one with a limited number of samples. Larger community based studies are warranted to bring out the actual magnitude of the disease.

CONCLUSION

Severe cases of haemolytic disease of newborn in Rh-D alloimmunisation is limited to <10% and after the advent of better neonatal care and monitoring services survival rates of affected newborns are much higher. An efficient monitoring system with simple laboratory parameters and imaging can help to bring down the adverse effects associated with Rh-D sensitisation in neonates.

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