Clinical and Immunohaematological Course of 64 Cases Of Antenatal Rh D Alloimmunisation At A Tertiary Care Centre In India

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Abstracts: Background and Objectives: Incidence and outcome of Hemolytic disease of Fetus and New-born due to RhD alloimmunisation has changed in last few decades after the advent of RhIG and other diagnostic and therapeutic tools. But reports from different centres vary. In this study Rh D sensitised antenatal women were followed up at Medical college, Trivandrum and clinical &laboratory profile analysed. Objectives of the study are to describe the clinical &laboratory profile of Rh D alloimmunised pregnant ladies and to describe severity and treatment of Hemolytic Disease in their off springs. **Materials and Methods:** Cross sectional study done on 64 antenatal cases, positive for anti Rh D antibodies by ICT and followed up with serial titres and ultrasound. Cord blood values and Direct Coombs test were used to diagnose HDFN at birth. Data was analyzed in SPSS ver.17.catagorical data was expressed in percentages and continuous data was expressed with mean and standard deviation. **Results**: Out of 2,496 Rh D negative women tested with ICT, 78 (3.12%) were positive.54 RhD positive new-borns were DCT positive (93.1%).50.9% cases were unaffected or mild. Severe cases accounted for 10% only. Majority (50%) received no treatment and phototherapy was the major modality of treatment. Overall survival rate of affected new-borns was 92.18%. Out of 6 hydropic babies, 4 died in utero. **Interpretations and Conclusions:** Rh alloimmunisation is still prevalent among antenatal women, but majority of cases produces only mild disease in new-born. Survival rate in newborns is >90%. Hydropic babies have a higher death rate. Better strategies to prevent Rh D alloimmunization and introduction of interventions like IUT are warranted. [Shaiji PS NJIRM 2014; 5(5):38-43]

Key Words: clinical course, Rh D alloimmunisation, antenatal women,hemolytic disease

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Introduction: Frequency of Rh D mediated HDFN has been reduced to around 0.2% of all Rh D negative women in developed countries after the lifesaving invention and implementation of RH Immunoglobulin[1].But it still remains as an important complication of pregnancy which needs to be anticipated and monitored in every Rh D negative woman. Effectiveness of prophylaxis depends upon the awareness and accessibilty of susceptible women to healthcare. Management of alloimmunisation once occurred is dependent on the facilities of monitoring as well as level of neonatal care available. Hence this study attempts to describe the clinical and immunohaematological profile of 64 alloimmunised antenatal women presented to a tertiary care centre in Trivandrum, Kerala, India.

Aim and Objectives:

- To describe the clinical &immunological profile of Rh D alloimmunised antenatal women.
- To describe severity and treatment of Hemolytic Disease in offsprings of alloimmunised women

Materials And Methods: This is a cross sectional study done in 64 Rh D negative antenatal cases found positive for anti Rh D antibodies from 1-10-2008 to 30-09-2010 in collaboration between Dept of Transfusion Medicine and Dept of obstetrics and gynaecology and was approved by an institutional review board and ethical committee. Informed consent was taken from all study subjects.

ABO grouping, Rh D typing, weak D test, antibody screening and identification with 3&11 cell panel (DiaPanel, M/sDiamed Inc,) were done on study subjects. Antibody titration was done on Indirect Coombs Test (ICT) positive samples by tube method. Serum preserved frozen at -20^{o} C for comparison. Patients were followed up monthly till 28 weeks and thereafter biweekly. Serial antibody titers 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 hemoglobin, DirectCoombsTest(DCT),

cord blood bilirubin and Peripheral smear of new-born also were tested. The incidence of DCT positivity and Hemolytic disease noted .Diagnosis of hemolytic disease of new-born due to Rh D alloimmunisation was considered if a positive DCT or positive eluate for anti D was found in an Rh D positive baby. Evidence of erythrocyte destruction like raised reticulocyte count, hemolytic picture in peripheral smear without any other possible cause for hemolysis was taken as HDFN even in absence of a positive DCT. Stillborn with clinical evidence of hydrops were also taken as affected. For diagnosis and treatment of hyperbilirubinemia chart by American Academy of Pediatricians was followed. Hemoglobin (Hb), bilirubin and reticulocyte count were done in 14 days in review clinic. Intra Uterine Deaths (IUD) in our study could be only clinically examined for evidence of hydrops and could not be autopsied due to refusal of relatives.

The severity of disease was graded as mild, moderate and severe[2].Mild cases were those with cord blood Hb values more than 12g% and bilirubin >3.5g%,moderate cases were those with cord Hemoglobin less than 12g% and bilirubin more than 3.5mg%). Hydrops and stillbirths were included in severe cases. Intensity of treatment was graded[3]. Grade 0 was those with no treatment needed, Grade 1 was treated with phototherapy alone, Grade 2 –phototherapy and IvIg,Grade 3 exchange transfusion& grade 4 -if multiple exchanges were performed.

Observations: Total number of Rh D negative women in whom ICT for anti D antibody was done in the Dept. of Transfusion Medicine from 1-10-2008 to 30-09-2011 were 2496.out of 78 positive cases,64 cases positive for ant D and were available for follow up in the hospital were included in the study. Characteristics of study subjects are described in Table 1**.**

Of the 2 cases of alloimmunisation in Primi which contributed to 3.13% of total alloimmunisations, one had a history of antepartum bleeding and other did not remember a relevant history.

40.6% (26)had a history of abortion in previous pregnancies.9.37% (6cases)had a history of IUD and14.06%(9 cases) had previous history of neonatal death.No invasive procedures like amniocentesis/chorionic villus sampling were done in any of the study subjects.

Failure to administer RhIg lead to alloimmunisation in 42 cases. In 14 cases, although RhIGwas administered postpartum, alloimmunisation occurred. 8 cases were not able to recollect previous history of immunization with RhIG.

No study subjects received antepartum RhIG. Maximum maternal titer ranged from 1 in 2 to 1 in 1024 in study subjects.

Table I: Characteristics of Rh D Alloimmunised Women

Age 18-23 yr 23-28 yrs 28-33 yrs 33-38 yrs 38-43 yrs	Number(% out of 64 alloimmunised patients) 15 (23.44) 29 (45.31) 13 (20.31) 6 (9.37) 1 (1.56)
Parity Primi 2nd gravidae 3rd gravidae 4th gravidae 5TH gravidae	 2 (3.13) 36 (56.25) 17 (26.56) 6 (9.38) 3 (4.68)
Order of affected pregnancy First Second Third Fourth	 46 (71.87) 12 (18.75) 3 (4.69) 3 (4.69)
Maximum maternal anti D antibody titre 2-8 8-32 32-128 128-512 512-1024 >1024	 22(34.37) 16(25) 14(21.9) 8(12.5) 3(4.68) 1(1.55)

Vaginal (19 spontaneous and 21 induced cases) constituted 62.5% (40) of total deliveries. 40 cases (62.5%) completed term and 31.25% (20%) were delivered preterm. Rest 4 (6.25%) died in utero. 36

babies (56.25%) showed adequate birth weight for gestational age (AGA). Evidence of Intrauterine growth retardation was present in 37.5%. Mean gestational age at delivery in our study was 251 days/35.6 weeks + SD 18.431.

3 out of New-borns were Rh D negative and 57 were Rh D positive. In 4 cases who died in utero,Rh D status was not assessed. Frequency of Hemolytic Disease of Fetus and New-born was 57 out of 64 cases.54 Rh D positive new-borns were DCT positive(93.1%) and 4 were DCT negative (6.9%). DCT positive infants were classified as affected and DCT negative as unaffected. Characteristics of new-borns are summarized in Table 2**.**

Of total alloimmunised pregnancies 82.82% turned out to be DCT positive. New-borns of alloimmunised mothers in our study were predominantly males (59.37%). Male: female ratio was 1.46:1.

DCT graded 2 in the majority of cases 29 cases (53.7%).Rest of the cases14 cases were unaffected (DCT negative) constituting 7% of newborns of alloimmunised mothers. 25 cases had a mild disease and required no treatment. 22 cases had moderate disease in which 4 required exchange transfusions. 6 cases developed hydrops in utero and were classified as severe. 2 cases who survived had ultrasound evidence of mild hydrops only, another 4 cases died in utero.

29 out of 58 live born new-borns needed no treatment. 15 were treated with phototherapy alone. 8 cases needed physiotherapy along with IVIg when phototherapy alone could not decrease the hyperbilirubinemia. 4 cases needed exchange transfusion. Out of 15 cases transfused with RBC, 11 cases needed only one dose of RBC transfusion and 4 needed multiple top up transfusions. Out of 54 affected (DCT positive) newborns only 4 (7.5%) needed exchange transfusion.

Table2:.Characteristics of New-borns born to Rh D alloimmunised mothers

New-borns	Number(percentage out of 64)
Rh D positive with positive DCT	53(82.82)
Rh D positive with negative DCT	4(6.25)
Rh D negative	3(4.28)
Still born	4(6.25)
DCT Grade in affected	
1	14(25.9)
2	29(53.7)
3	3(5.6)
4	8(14.8)
Sex	
Males	38 (59)
females	26(41)
Disease Severity in Rh positive babies	4(7)
Unaffected	25(43.9)
Mild	22(38.6)
Moderate	6(10.5)
Severe	
Treatment	
No treatment	29(50)
Phototherapy	15(25.9)
Photo+Ivlg	8(17.2)
Exchange transfusion	4(6.9)
Survival	
Intrauterine death	4(6.25)
Livebirth	59(92.18)
Neonatal death	1(1.5)

Overall incidence of exchange transfusion in 64 cases studied were 6.25%.8 cases needed Ivlg along with phototherapy and 2 cases received Ivlg along with phototherapy and exchange. Ivlg was not given without phototherapy in any case.

Of 48 infants whose hemoglobin levels were examined in review clinic, 2 had anemia and minimal jaundice requiring re-admission. 46 infants had normal hemoglobin and bilirubin levels. Remaining 12 cases did not attend the review clinic at 2 weeks.

59 of 60 live born infants survived. The overall survival rate of new-borns in 64 alloimmunised pregnancies was 92.18%.The survival rate in live born was 98.36% .

Statistical analysis: Categorical variables were expressed as percentage and continuous variables

were described as mean and standard deviation. Comparison between groups were done by independent 't' test.

Discussion: The frequency of anti D antibodies detected in our study is 3.125% of all Rh D negative women. According to global statistics, rate of Rh D alloimmunisation is reported to be 0. 2% in centres with routine antenatal Rh Ig prophylaxis and 1-2% in centres with only postpartum prophylaxis[1]. The prevalence reported by various studies follow a wide range from .2 to 6%[4,5] but most of the studies report a prevalence less than <2%. Slightly higher prevalence in our study may be due to referred Rh D negative cases from peripheral centres for better antenatal and neonatal care. Since this was not conducted as a community based prevalence study actual prevalence cannot be commented upon.
Around 2/3rd of our study subjects are less than 30 yrs of age which may parallel the child bearing age distribution of women in India. The majority of sensitized cases were reported in the second pregnancy.

We found 2 cases who were alloimmunised in first pregnancy itself. One had a history of antepartum bleeding at 28th week where as others could not give a history of any suspected risk factors like APH or invasive procedures/previous transfusion with Rh D positive blood.

Frequency of alloimmunisation in primi as observed in our study is 3.12% of alloimmunised women. Our study could not provide an incidence in primi since a statistics regarding the parity of all the Rh D negative women was not taken.

We observed that 65.6% cases of alloimmunisation were due to lack of Rh D prophylaxis. In 21.9% of cases even though Rh D prophylaxis was given it was not effective in protecting the woman against immunisation. Rest of the cases could not remember and did not have any records of history of immunisation. It emphasizes that measures for improving the vaccination programme for women should be sought for in the state.

Decision for preterm delivery depends upon the severity of disease and neonatal care facilities. Similarly, mode of delivery depends on maturity of fetus, need for intervention which can be emergency or elective and mainly obstetric considerations. According to our study 62.5% of the cases were vaginal deliveries and rest had undergone Lower segment caesarian section (LSCS). The frequency of premature deliveries was 31.25%. A study done in New Delhi, India[6] reported Caesarian section as commoner mode of delivery (67.5%) in affected children where as some reported more of vaginal deliveries.

Mean gestational age at delivery in our study was 251 days (35 weeks 6 days + SD 18.431 which is higher than that reported by Trainer and Tubman[7] which is 31.5 (Range 26-38) weeks but subjects were more seriously affected there. Our results are similar to an Indian study which observed mean gestational age of 35-36 weeks in babies who were not given IntraUterine Transfusion (IUT) [6]. It is obvious that mean gestational age at delivery varies in centres who practice IUT and those who do not.

It is postulated that D positive infant that initiates Rh D immunization is more frequently male than female[8,9]. Ratio found in our study was 1.46:1.

The frequency of affected children found in our study was 57 out of 64 alloimmunised cases constituting 2.28% of newborns born to Rh D negative women examined. Many authors have reported that more than half of the new-borns of alloimmunised mothers are either unaffected or mildly affected [6,10].In our study 7% were unaffected and 43.9% were mildly diseased together constituting a 50% and severe cases going into hydrops were 10%. Intra uterine transfusion was not given to any because the facility was not available.

Treatment outcomes of Rh D-positive pregnancies in sensitized women as in a study by Bowman et al in 1978 showed 29.4% incidence of exchange transfusion. Later Filbey[11] et al reported 46% of all Rh-positive babies having phototherapy and 29% having exchange transfusion. A more recent study reported 7 exchange transfusions out of 26 affected cases[6]. With the advent of specific guidelines and advance options like IVIg the incidence of exchange transfusion is found to be

decreasing. In our study majority of cases required no treatment, Exchange transfusion was done in much lesser number of new-borns 4/64 (6.25%) and phototherapy was the main mode of treatment with additional IvIg in some cases.

The combination of Rh-D negative mother and Rh-D positive child was found in around 3/4th of alloimmunised pregnancies in a study by Bowell[12] et al. Another study [13] found 3/19 alloimmunised cases (15.78%) as Rh D negative. We have observed Rh D negative blood group in 5% offsprings of alloimmunised mothers and the rest were Rh D positive.

DCT in offsprings of Rh D alloimmunised mothers were found positive in 82.82% infants of alloimmunised mothers in our study. Survival above 92% was reported in our study similar to many studies [6,10]But the availability of therapeutic measures like Intrauterine Transfusion could have improved the outcome since 4 cases of intrauterine deaths were observed. One new-born in our study had severe thrombocytopenia with gastrointestinal bleeding manifestations. She survived but had to undergo multiple platelet and RBC transfusions.

Descriptive nature of the study does not allow for testing associations with HDFN. An analytical study with larger sample size is warranted.

Conclusion: Rh D alloimmunization and HDFN still occurs in the study setting but with a good survival. Interventions on pre-hydropic and hydropic babies should be focused more because mortality is mainly in that group. Attempts to increase RHIG immunization rates would be worthwhile.

Conflict of interest statement
Conflict of interest-None

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