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Neonatal Outcomes of Rh-Negative Pregnancies in a Tertiary Level Neonatal Intensive Care Unit: A Prospective Study

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Abstract

Background: Rhesus incompatibility is a preventable cause for severe neonatal hyperbilirubinemia, hydrops fetalis and still births. The prevalence of the Rh-negative blood group among Indian woman varies from 2% -10%. Despite declining the incidence of Rhesus incompatibility, due to availability of anti-D immunoglobulin, and improved antenatal care of the Rh-negative pregnant woman, it still accounts for a significant proportion of neonatal hyperbilirubinemia and neuro-morbidity. The prevalence of Rh-negative women having Rh-positive neonates is 60%.

Objectives: This study aimed to estimate the incidence of Rh iso-immunization and evaluate the outcomes of Rh iso-immunized neonates.

Methods: This prospective observational study was conducted in a tertiary level neonatal intensive care unit, Princess Esra hospital, Deccan college of medical sciences, Hyderabad, Telangana, India. Consecutive intramural and extramural neonates admitted to neonatal intensive care unit with the Rh-negative mother's blood group and hyperbilirubinemia were enrolled. Neonates born to Rh+ve mothers were excluded. Neonatal gestational age, birth weight, age at admission, duration of phototherapy, duration of hospitalization, neonatal examination and investigations were recorded in a predesigned, pretested performa.

Results: A total of 90 neonates were born to Rh-negative mothers, of which 70% (63) had the Rh-positive blood group and 30% had the Rh-negative blood group. Of these 63 neonates, 48 (76.2%) had hyperbilirubinemia and 43 neonates (68.3%) had significant hyperbilirubinemia (total serum bilirubin > 15mg/dL). Among them, 2%, 75% and 23% were born to primi, multi and grandmutli, respectively. Also, 14.5% of the neonates were large for dates (LFD), 75% appropriate for dates (AFD) and 10.5% were small for dates (SFD). Premature and SFD neonates had higher incidence of hyperbilirubinemia. Significantly higher incidence of jaundice occurred within 72 hours of life. The mean serum bilirubin level among neonates with significant hyperbilirubinemia was 17.98 $\pm$ 1.76 (95% CI:17.43 - 18.52) while that of neonates without significant hyperbilirubinemia was 13.1 $\pm$ 0.53 (95% CI:12.47 - 13.77) with statistical significance ($P < 0.0001$). Maternal multiparity, direct coombs test (DCT) positivity, and abnormal neurosonogram (NSG) were important accompaniments of high serum bilirubin levels.

Conclusions: Incidence of Rh-positive phenotype in neonates born to Rh-negative mothers is 70%. Incidence of hyperbilirubinemia among them is 76.2% and that of significant hyperbilirubinemia was 68.3%. The incidence of DCT positivity was 20.9%. Maternal multiparity, positive DCT and abnormal NSG were important associates of high serum bilirubin levels.

Keywords: Neonatal Hyperbilirubinemia, Rh-isoimmunization, Direct Coombs Test, Anti-D, Multi Parity, Serum Bilirubin

1. Background

Rhesus incompatibility has been an important cause for severe neonatal hyperbilirubinemia, hydrops fetalis and still births since 1939 (1, 2). Although, its incidence declined steadfastly since late 1960s with the introduction of anti-D immunoglobulin, it still continues to be a major determinant to kernicterus and extra pyramidal cerebral palsy (1-3). The reported incidence of Rh-negative women having Rh-positive phenotype babies is 60% (4, 5). Administration of Rh immunoglobulin to Rh (D) negative women during antenatal period and soon after the birth of the Rh (D) positive infants has lead to reduction in the incidence of maternal D alloimmunization from 14% to 1%. However, this pathology continues to occur in 0.4 of 1,000 births (0.04%) for multiple reasons like lack of anti-D immunization during the pregnancy (about 1%), ineffec-

tive immunoprophylaxis (insufficient dose of anti-D immunoglobulin for the degree of the fetal-maternal hemorrhage), and possible errors in Rh typing of the pregnant or postnatal woman or the neonate. The magnitude of the hemolytic disease of fetus and neonate (HDFN) is influenced by the immunoglobulin subclass, antibody levels and number of antigenic sites on the red cell (1). Paucity of data regarding Rh isoimmunization from developing countries has led to the present study (6, 7).

2. Objectives

This study aimed to estimate the incidence of Rh isoimmunization and evaluate the neonatal outcomes and their determinants.

3. Methods

This prospective observational study was conducted in a tertiary level neonatal intensive care unit (NICU), Princess Esra hospital, Deccan college of medical sciences, Hyderabad, Telangana, India, over a period of 6 months (January 2015 - June 2015). There was no separate control group, but neonates without hyperbilirubinemia worked like an auto control group for comparisons.

Consecutive neonates born to Rh-negative mothers having hyperbilirubinemia were enrolled. Neonates born to Rh-positive mothers were excluded from the study.

Neonatal gestational age, birth weight, age at admission, duration of phototherapy, duration of hospitalization, neonatal examination and investigations were recorded in a predesigned pretested performa.

3.1. Sample Size Calculation and Statistical Analysis

As it was a pilot study in this NICU, a convenient sample size of 100 was chosen. However, only 90 mothers with the Rh-negative blood group participated in the study. Online statistical software (GraphPad) was used to calculate means $\pm$ SD, and 95% CI. Fisher's exact test and t-test were also used to analyze the data. P value of < 0.05 was considered as statistically significant.

3.2. Ethical Approval

the ethical approval was obtained from institute's ethics committee. No extra investigations were done for the study purpose.

4. Results

A total of 90 neonates were born to mothers with Rh-negative blood group during the study period, of which 27 (30%) neonates had Rh-negative blood group and 63 neonates (70%) had Rh-positive blood group. Thus, the incidence of Rh-positive phenotype neonates to Rh- negative mothers is 70%. Hence, these 63 neonates with Rh-positive blood group were evaluated. Out of them, 5 cases (7.9%, 5/63) were born to primipara without previous sensitization. However, 46 cases (73%) were born to multipara and 12 (19%) were born to grand multiparous mothers.

From a total of 48(76.2%) neonates with hyperbilirubinemia, one was born to primi, and 47 (74.6%) neonates were born to mutli and grandmulti. When parity was evaluated as a risk factor for hyperbilirubinemia in Rh-negative pregnancies, grandmultiparous mothers had the highest incidence of hyperbilirubinemia (11/12, 91.7%) with statistical significance (P: 0.01), as shown in Table 1.

When gestational age was evaluated as a risk factor, 75.5% of term neonates, 80% of late preterms and extreme premature neonates had hyperbilirubinemia. Among the neonates with hyperbilirubinemia, seven neonates were large for dates (LFD), 36 were appropriate for dates (AFD) and the rest of the five neonates were small for dates (SFD). The mean birth weight of study population was 2833.33 $\pm$ 565 (95% CI: 2669.15 - 2997.52). Hyperbilirubinemia was high among SFD neonates (83.3%), when compared to other groups. Also, 12.5% of the neonates had total serum bilirubin levels (TSB) levels > 20mg/dL, and10.4% had TSB between 12 and14.9 mg/dL. Most of them had TSB levels between 15 and 19.9mg/dl (as shown in Table 1). The mean serum bilirubin level in neonates with significant hyperbilirubinemia (TSB > 15mg/dL) was 17.98 $\pm$ 1.76 (95% CI:17.43 - 18.52). However, the mean serum bilirubin level in neonates with mild hyperbilirubinemia was 13.12 $\pm$ 0.53 (95% CI:12.47 - 13.77). When both mean values were compared, the difference was 4.88 mg/dL (95% CI- 3.26 - 6.51) which was statistically significant (two-tailed P < 0.0001). Significantly a higher proportion of neonates with Rh incompatibility had hyperbilirubinemia within 72 hours of life ($P < 0.001$). Thus, neonates with Rh isoimmunization had significantly higher incidence of jaundice within 72 hours of life (as shown in Table 1). The direct coombs test (DCT) was performed in 43 neonates with TSB > 15mg/dL and nine neonates had features of hemolysis in the form of anemia, and hepatosplenomegaly along with positive DCT. Thus, the incidence of Rh isoimmunization was 20.9% (9/43) and that of positive direct coombs test was 20.9% (9/43). The direct coombs test was not performed in neonates with TSB < 15mg/dL, as they did not have features of hemolysis and Rh-iso immunization. Sig-

Table 1. Incidence of Jaundice

Parameter	Mean Value ± SD	N	Percentage, %	95% CI	P Value
Mean TSB					< 0.0001
Infants with significant hyperbilirubinemia	17.98 ± 1.76	43	89.6	17.43 - 18.52	
Infants with mild hyperbilirubinemia	13.12 ± 0.53	5	10.4	12.47 - 13.77	
Difference in mean of both the groups	4.88	-	-	3.26 - 6.51	
Birth weight, g	2833.33 ± 565	48	100	2669.15 - 2997.52	
Mean duration of phototherapy, h					< 0.0001
Significant hyperbilirubinemic infants With IVIG	60.12 ± 12.31	20	46.5	54.24 - 65.76	
Without IVIG	93.57 ± 10.21	23	53.5	89.2 - 98	
Difference between both the groups	33.57	-	-	40.52 - 26.63	
Mean duration of hospitalization, d					< 0.0001
IVIG group	7.81±1.58	20	46.5	7.11 - 8.54	
No IVIG group	12.96 ± 1.66	23	53.5	12.24 - 13.7	
Difference between both the groups	5.16 days			6.21 to - 4.21	
Parity					< 0.01
Primi with hyperbilirubinemia	-	1	20		
Multi with hyperbilirubinemia	-	36	78.3		
Grand multi with hyperbilirubinemia	-	11	91.7		
Severity of hyperbilirubinemia					< 0.001
> 20, mg/dL		6/48	12.5		
15 - 19.9, mg/dL	-	37/48	77.1	-	
12 - 14.9, mg/dL	-	5/48	10.42	-	
Age wise distribution of hyperbilirubinemia					< 0.001
< 24, h		4/5	80		
24 - 72, h	-	32/34	94.12	-	
> 73, h	-	12/24	50	-	
Correlation of TSB levels with DCT					< 0.001
TSB > 20, mg/dL		5/6	83.33	-	
TSB:15 - 19.9, mg/dL	-	4/37	10.81	-	

nificantly a higher proportion of neonates (5/6, 83.3%) with DCT positivity had TSB > 20 mg/dL (P < 0.001).

Neurosonogram (NSG) was performed in neonates with TSB levels > 15 mg/dL, by a single sonologist (to avoid interobserver variation). This group was chosen as these neonates are more likely to have neurological impairment. Neurosonogram findings were abnormal with hyperintense basal ganglia in 20.9% (n = 9). There was a significant association between abnormal NSG and high bilirubin levels (P < 0.001) and two of these neonates with abnormal imaging had clinical evidence of kernicterus. Therefore, the incidence of kernicterus in this study was 3.2% (2/63).DCT positivity was significantly higher among the neonates born to grand multiparous mothers (two-tailed P < 0.001). Significantly a higher proportion of neonates (80%) born to grand multi had bilirubin levels > 20mg/dL. Exchange transfusion (ET) was required for 4 (9.3%) neonates with TSB > 20mg/dL, of which 1 neonate had TSB > 22mg/dL and required ET twice. Intravenous immunoglobulins were given to 20 neonates (46.5%) at 1g/kg/dose for 2 consecutive days, of which 5 required IVIG twice. Eighty-six percent of multiparous mothers received anti D. Ninety percent received RhoGAM, while the rest received Rhoclone. The indirect Coombs test (ICT) was positive in 9 mothers and the neonates born to them had positive DCT. Late onset anemia was detected in 7(16.3%)

neonates and all of them had features of congestive cardiac failure, requiring top-up transfusions. None of the neonates in the study population died. Mean duration of phototherapy in neonates who received intra venous immunoglobulin was 60.12 ± 12.31 hours (95% CI: 54.24 - 65.76 hours). While the mean duration of phototherapy in neonates who did not receive intravenous immunoglobulin was 93.57 ± 10.21 hours (95% CI :89.2 - 98 hours). When both means were compared, the difference was 33.57 (95% CI-40.52 - 26.63) and this difference was statistically highly significant (P< 0.0001). Similarly, mean duration of hospitalization in neonates who received intravenous immunoglobulin was 7.81 ± 1.58 days (95% CI:7.11 - 8.54 days) and that in neonates who did not receive intravenous immunoglobulin was 12.96 ± 1.66 (95% CI: 12.24 - 13.7). The difference between both groups was -5.16 days (95% CI:-6.2 to -4.2), which was statistically highly significant (two tailed P < 0.0001). Three neonates with TSB levels > 20mg/dL had abnormal antenatal Doppler of middle cerebral artery (MCA) velocity. None had hydrops fetalis and none received intrauterine transfusion. Only four multipara mothers had a history of stillbirths although its exact incidence could not be obtained.

5. Discussion

The proteins carrying the Rh antigens are transmembrane proteins, structurally similar to ion channels. Either placental sensitization or transfusion leads to the development of antibodies against Rh factor (1). Rh antigens are located only on red blood cells (RBC) and RhO (D) antigen is the most important antigen in the Rh system, as it is highly immunogenic. Asians are reported to have less than 1% of the RhD-negative blood group and alleles (1). Red blood cells lacking Rh antigens are known to have an abnormal shape, increased osmotic fragility and thus, shortened life span leading to hemolytic anemia. Despite tremendous advances, the incidence of Rh alloimmunization remains as an important cause for neonatal hyperbilirubinemia, more so in the developing countries with limited anti D availability. A study from west Bengal reported an incidence of 2.4% Rh-negative woman (16/657) (4). Although it is less than 10%, a neonate with isoimmuzation is a medical emergency (1-4). Transplacental passage of maternal antibodies in Rh isoimmunization results in the hemolytic disease of fetus and neonate (HDFN) due to immune hemolysis of fetal or neonatal RBC5. This leads to progressive anemia coupled with hypoalbuminemia and fetal heart failure (hydrops fetalis), an important cause for still births and early neonatal deaths. Those who survive can manifest with hyperbilirubinemia, anemia, kernicterus and neonatal morbidity and mortality (6, 7).

Rh D is the most potent immunogen with even 0.1 to 1 mL of Rh D positive red cells stimulating antibody production (5, 8). The identification of antibodies in the mother predicts the potential risk for HDFN and Rh-negative women with initial absence of antibodies requires prophylaxis. When prophylactic Rh IG is administered, anti D can be detected up to 8 weeks by an indirect agglutination test/indirect coombs test [IAT/ICT]). Similarly, immune anti D becomes detectable by 4 weeks after exposure to D positive cells reaching its peak by 6 - 8 weeks. Prophylactic anti D levels fall with time while immune anti D levels remain stable or rise, differentiating both conditions (8). Serial determination of antibodies coupled with medical history enables identification of either immune or prophylactic anti D. The antenatal screening protocol recommends all pregnant women to get tested for ABO, Rh D type along with a red cell antibody screen at 12 weeks of gestation (9). When significant antibody titers are detected, periodic monthly retesting till 32 weeks, followed by twice weekly till term are recommended. If alloantibodies are absent in the first antenatal visit, screening needs to be repeated at 28 - 32 weeks and no further screening is needed if the titers are negative (10). If a woman receives anti D injection, sampling should be done prior to the anti D injection.

In the present study, 70% of mothers had Rh-positive neonates, which is comparable to previous study by Agarwal et al. (1). In the present study, the anti D coverage was 86% and the majority received it within 24 hours after the delivery of an Rh-positive neonate. Cochrane review shows that antenatal prophylaxis [100 μg, 500 UI of anti-D IgG at 28th to 34th weeks] reduces the risk of alloimmunization of Rh(D)-negative pregnant women from 1% to 0.2% along with the reduction in the immunization of subsequent pregnancies. Number needed to treat (NNT) to prevent one case of sensitization is 213 (5). However, literature reports that 1.8% of Rh (D)-negative women continue to produce anti-D antibodies despite postnatal prophylaxis, due to small transplacental hemorrhages during pregnancy 5.This could be tackled by anti D administration during pregnancy.

A multicentric systematic review by Bhutani et al. (7) reported 0.36% of Rh disease with 24% risk for mortality, 13% risk for kernicterus and 11% for stillbirths. Three- quarters of mortality was reported from sub-Saharan Africa and South Asia. Prevalence of kernicterus due to Rh disease from South Asia is 28 per 100000 live births. Majority of neonates with kernicterus had impairments. Countries with neonatal mortality rate (NMR) < 5 had good Rh prophylaxis and less Rh disease (7). It has been reported that, 15% of women with previous sensitization develop Rh isoimmunization. An estimated prevalence for South Asia is 385 per 100000 in contrast to that of developed countries

with a prevalence of 2.5 per 100000 live births. They reported a prevalence of Rh disease as 277/100,000 live births. Most of the Rh disease and extreme hyperbilirubinemia occurred in countries with NMR > 15, which accounts for 60% of the global live births. Walker et al. (11) in their large series of Rh pregnancies reported that 33% of the neonates required no treatment. Also, in the current study 23.8% of the neonates did not require any treatment. Hsia et al. (12) reported that 50% of neonates with peak TB > 30mg/dL, when untreated developed kernicterus, in par with observations reported by Mollison and Walker (13). In the current study, only two neonates had extreme hyperbilirubinemia (TSB > 22mg/dL) requiring ET and had kernicterus and abnormal NSG. The incidence of kernicterus was 3.2%. Although this incidence rate is much less than the literature, it leads to permanent impairment in those children. The goal of optimum neonatal care includes prevention of kernicterus. These neonates in the present study were outborns and had features of kernicterus prior to the admission to the NICU and could not be reverted with exchange transfusion, as they had stage II kernicterus. However, they were under periodic neurodevelopmental follow-up and MRI imaging. The incidence of Rh isoimmunization has been reported to be 13% by Freda et al. (14-19) and the current study found an incidence of 14.3%,which was comparable. Also, 46.5% of the study neonates received IVIG and had showed decline in the bilirubin levels with immunoglobulin therapy. Mean phototherapy duration, and mean duration of hospitalization were significantly less in neonates treated with IVIG compared to other neonates. A randomized controlled study by Girish et al. from all India institutes of medical sciences, New Delhi, reported that neonates receiving 1g /kg of IVIG had shortened duration of phototherapy (55.4+/-49 hours in a high-dose group) when compared to those who received 0.5 g/kg (77.3+/-57.2). There was no significant difference in the duration of hospitalization between both the groups (8.4+/-6.9 and 13.6+/-14.8 days). In this study, only high-dose IVIG (1 g/kg/dose) was used with significant reduction in duration of phototherapy and hospitalization. Also, the mean duration of phototherapy and hospitalization in the IVIG group of the current study is comparable to the high- dose IVIG group of Girish et al. study (20). Similarly, IVIG has also been used in Iran in HDFN (due to Rh isoimmunization) to reduce the need for exchange transfusion (14). Immunoprophylaxis both during antenatal period as well as postnatal remains the prime mode of prevention of Rh disease especially in resource poor settings where intrauterine transfusions are not available, one like the present study. The anti D immunoprphylaxis was quite good in the present study, probably contributing to fewer incidences of Rh isoimmunization and kernicterus. Only 6.9% of the multipara mothers had still births. Presence of high serum bilirubin levels, DCT positivity and abnormal NSG in neonates born to grand multiparous mothers indirectly suggest their sensitization in previous pregnancies. As nations with higher NMR ($\geq$ 15) are at the greatest risk for neonatal mortality from Rh disease and EHB, prevention of Rh sensitization and optimum care of every neonate along with management of extreme hyperbilirubinemia is a vital implementation priority.

5.1. Conclusions

The incidence of Rh-negative mothers having Rh-positive phenotype neonates was 70%. The incidence of hyperbilirubinemia among them is 76.2%. Neonates born to grand multiparous mothers had highest incidence of hyperbilirubinemia (91.7%). Hyperbilirubinemia was high among preterm and SFD neonates. Most of the neonates (77.1%) had bilirubin levels between 15 mg/dL and 19 mg/dL. Overall incidence of Rh isoimmunization was 14.3% and DCT positivity was 20.9%. It was significantly high among the neonates born to grand multiparous mothers. A significantly higher proportion of neonates with DCT positivity had severe hyperbilirubinemia (TSB > 20 mg/dL).Anti D coverage for Rh-negative pregnant woman was 86%. The incidence of kernicterus was 3.2%. There was a significant association between high bilirubin levels and abnormal NSG (83.3% of the neonates with TSB > 20mg/dL had abnormal NSG). Also, 46.5% of the neonates received a high dose IVIG treatment with significant reduction in mean phototherapy duration and hospitalization in the IVIG group. Maternal multiparity, positive DCT and abnormal NSG were important correlates of high serum bilirubin levels.

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