RESEARCH LETTER

Neurosonographic Findings in Infants with Rhesus Hemolytic Disease of Newborn: A Prospective Observational Study

Swapna Bayye[1], Jogender Kumar[1], Subhas Chandra Saha[2], Sourabh Dutta[1], Praveen Kumar[1]

Department of [1]Pediatrics and [2]Obstetrics and Gynecology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Correspondence to: Dr. Jogender Kumar, Neonatal Unit, Department of Pediatrics; Postgraduate Institute of Medical Education and Research, Chandigarh, 160012 India. *jogendrayadv@gmail.com*

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ABSTRACT

We estimated the incidence of intraventricular hemorrhage (IVH) and/or periventricular leukomalacia/echogenicity (PVL/E) in Rhesus isoimmunized infants. Seventy-one infants underwent cranial ultrasound within the first 3 days of life or discharge, whichever was earlier. Of these, 27(38%) infants had IVH/ PVL/E. On multivariate analysis, lower gestational age ($P = 0.035$), small for gestational age [aOR (95% CI) 10.6 (1.9, 58.9)], and sepsis [aOR (95% CI) 4.5 (1.1, 18.4)] were independently associated with IVH/PVL.

Keywords: *Anemia, Cranial ultrasound, Intraventricular hemorrhage, Neurodevelopmental outcome*

Infants born to women with Rhesus (Rh) isoimmunization during pregnancy are at risk for fetal anemia. Many of them undergo multiple intrauterine transfusions (IUTs) from the second trimester onwards. In-utero anemia coupled with hemodynamic fluctuations associated with IUT may increase the risk of fetal brain injury [1,2]. A few cohort studies have shown an increased incidence of intraventricular hemorrhage (IVH), cerebellar hemorrhage, and periventricular leukomalacia/echogenicity (PVL/E) following IUT in the fetal period [2–4]. There is very limited data on the neurosonographic abnormalities in the neonatal period [1,5], although the prevalence of IVH/PVL in infants with IUTs has been reported to vary from 35 to 57% [1,5].

Approximately 45% of infants born to Rh isoimmunized women in our unit require IUT. Assuming an incidence of IVH/PVL of 25% (50-55% of enrolled will be without IUT) and a 10% margin of error, a sample size of 72 participants was needed. The objective of this prospective observational study was to estimate the incidence and determine the predictors of IVH and/or PVL/E in Rh-isoimmunized infants.

Infants born between July 2020 and December 2022 to women with Rh isoimmunization and indirect coomb's test titers ≥1:16 were enrolled, irrespective of IUT status. Infants born to mothers with pregnancy-induced hypertension, chorioamnionitis, and immune thrombocytopenic purpura were excluded. Ethics approval and written informed parental consent were obtained.

Fetal anemia and the need for IUT were assessed per standard parameters during pregnancy [1]. Fetuses with middle cerebral artery peak systolic velocity > 1.5 multiples of the median were considered to have moderate to severe fetal anemia. They underwent IUT and invasive fetal blood sampling. In cases of IUT, pre-IUT fetal hematocrit of < 30% was considered anemia, and < 21% severe anemia [1]. Structured cranial ultrasound (CUS) was performed within the first three days of life or discharge, whichever was earlier [1,6]. The ultrasound scans were performed by pediatric radiologists, except in neonates admitted in the neonatal intensive care unit (NICU), where a trained neonatologist did the CUS using a multifrequency (4-12 MHz) phased array probe. Infants with abnormal CUS findings in the first instance were re-evaluated on day 7 of life. Periventricular echogenicity (PVE) was defined as confluent areas of increased echogenicity (iso/hyperechoic in comparison to the choroid plexus in the periventricular region) observed in the coronal and sagittal planes. Persistent flare/PVE for ≥7 days was considered abnormal and was labeled as PVL. PVL and IVH were classified according to de Vries and Volpe's classification, respectively [7]. Hearing screening was performed before discharge using automated auditory brainstem response (AABR).

We described categorical variables as percentages, normally distributed numerical variables as mean (SD), and those with skewed distributions as median (1st, 3rd quartile). We determined skewness by the Shapiro-Wilk test. We assessed the relationship between the aforementioned risk factors and the abnormal CUS findings using chi-square/Fischer exact tests for categorical outcomes and Mann Whitney-U test/Student's t-test for continuous outcomes, respectively. *P* value <0.05 was considered statistically significant. Variables with *P* value ≤ 0.1 on univariate analysis and meeting prerequisites (independence, linearity, normality, and homoscedasticity) for multivariate logistic regression were forced into the model (Enter method) for binary logistic regression. The Hosmer-Lemeshow test was used to assess the overall fit of the logistic regression model. The model's discriminative ability was evaluated using concordance statistics (Cstatistics). SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.) was used for analysis.

Seventy-four infants were eligible; three died before performing CUS, and 71 infants were included in the analysis. Of these, 31 (44%) received one or more IUTs. Twenty-seven (38%) infants had IVH (*n* = 23), PVL (*n* = 17), or both (*n* = 13). Most of the IVH were mild (Grade I = 12, Grade II = 10) except one infant who developed extensive periventricular hemorrhagic infarction (PVHI). Similarly, most had grade I (*n* = 12) or grade II (*n* = 5) PVL. There was no significant difference in the incidence of IVH/PVL among 15 (56%) infants who received IUT compared to the 12 (44%) who did not (*P* = 0.1). Two infants (one with PVHI and another with severe sepsis) died before discharge. None of the enrolled infants had cerebellar hemorrhage or significant structural anomalies. All had normal hearing on automated auditory brainstem response (AABR).

The profile of risk factors of IVH/PVL is given in **Table 1**. On univariate analysis, lower gestation, lower birthweight, ≥3 IUTs, lower gestational age at first IUT, perinatal asphyxia, neonatal sepsis (early-onset), and hypoglycemia were significantly associated with IVH/PVL. On multivariate analysis, lower gestation, small for gestational age (SGA) status, and neonatal sepsis were independently associated with the outcome. The multivariate regression model was a good fit (*P* = 0.2). The area under the curve (AUC, 95% CI) assessed by Cstatistics for the model was 0.85 (0.70, 0.99, *P* = 0.001), suggesting that the model has excellent discriminatory ability (**Fig. 1**). Among those who received IUTs, receipt of multiple IUTs (≥ 3) was independently associated with IVH/PVL.

In this study, more than one-third of infants had IVH or PVL or both, though most were mild. Prematurity, SGA, and neonatal sepsis were independent predictors for IVH/PVL. Among those who received IUT, receipt of ≥ 3 IUTs was associated with an increased risk for cranial abnormalities. We did not observe a significant association between the severity of anemia and IUTs with IVH/PVL.

Published data on brain injury in Rh-isoimmunized infants is limited. We could identify only two cohort studies on reviewing the literature [1,5]. Leijser et al. studied 127 neonates who received IUT [5] and observed CUS abnormalities in 57%, most of which were mild. The higher prevalence in this study than ours might have been due to earlier scanning (median 2 days) and possible inclusion of transient PVE. Sanchez-Duvan enrolled 41 neonates who received IUTs and screened them with CUS and/or magnetic resonance imaging (MRI) [1]. Cranial abnormalities were observed in 14 (35%), most of which were also mild. Leijser et al. assessed neurodevelopmental outcomes in the neonatal period and at two years of age, whereas Sanchez-Duvan followed up the infants for neurodevelopmental assessment until a median age of 6.5 years (range, 3

months to 19 years) [1,5]. In both studies, the cranial abnormalities were not associated with adverse neurodevelopmental outcomes [1,5].

In this study, the infants were mostly late preterm and hence not considered to be at risk for IVH or PVL (in the absence of other obstetric risk factors) [8]. Previous studies have reported that hypoxia/ischemia secondary to fetal anemia predisposes to PVL in fetal life. It is hypothesized that hyperdynamic circulation due to fetal anemia might injure the blood vessels in the immature brain with an increased predisposition to bleeding. IUT leads to rapid hemodynamic changes by acute volume overload coupled with a rise in hematocrit (and viscosity) in a relatively short period, further predisposing for bleeding [2,3,5]. Anemia and hypoxia are thought to be one of the risk factors for adverse neurodevelopmental outcomes among Rh isoimmunized infants [9]. The LOTUS study is the largest cohort study that enrolled 451 anemic fetuses who received IUTs and followed them until 17 years of age for neurodevelopmental outcomes [9]. These children (n=291) were assessed between 2-17 years of age (median 8.2 years) for physical, neurological, and cognitive development using age-appropriate standardized tests. In this study, all infants were anemic, but >95% had normal neurodevelopmental outcomes, similar to the general population. Anemia and IUT were not associated with poor neurodevelopmental outcomes. Like this study, Leijser et al. found no relationship between IVH/PVL, anemia, and IUT [5]. Though theoretically, it is possible that fetal anemia/IUT may make the fetus/neonate vulnerable to brain injury, while their presence or severity may not have a direct effect on the occurrence or severity of IVH/PVL [5,9,10]. However, the data is insufficient to establish or refute this hypothesis. There is a need for additional exploratory studies for better insight.

There are certain limitations in this study. We did not perform any fetal ultrasound; therefore, whether these findings were present even before the IUT is uncertain. As the first ultrasound was done within the first 72 hours, most findings (at least PVL) were presumed to be of fetal origin, consistent with previous studies. As the event rate for IVH/PVL was less than ideally required for the predictor variables chosen in this study, the results on predictors should be interpreted cautiously. We did not have long-term follow-up data; hence, the relationship between CUS abnormalities and neurodevelopmental outcomes could not be ascertained.

We conclude that more than one-third of infants born to Rh isoimmunized mothers had IVH and/or PVL in the first 3 days of life. Further larger studies beginning from the fetal period (to determine the timing of occurrence) are required to estimate the true incidence and predictors for cranial abnormalities. Long-term follow-ups to determine the association of the milder abnormalities with neurodevelopmental outcomes would be invaluable.

Ethics clearance: PGIMER Institute ethics committee approved the study (INT/IEC/2020/SPL-521) dated April 22, 2020.

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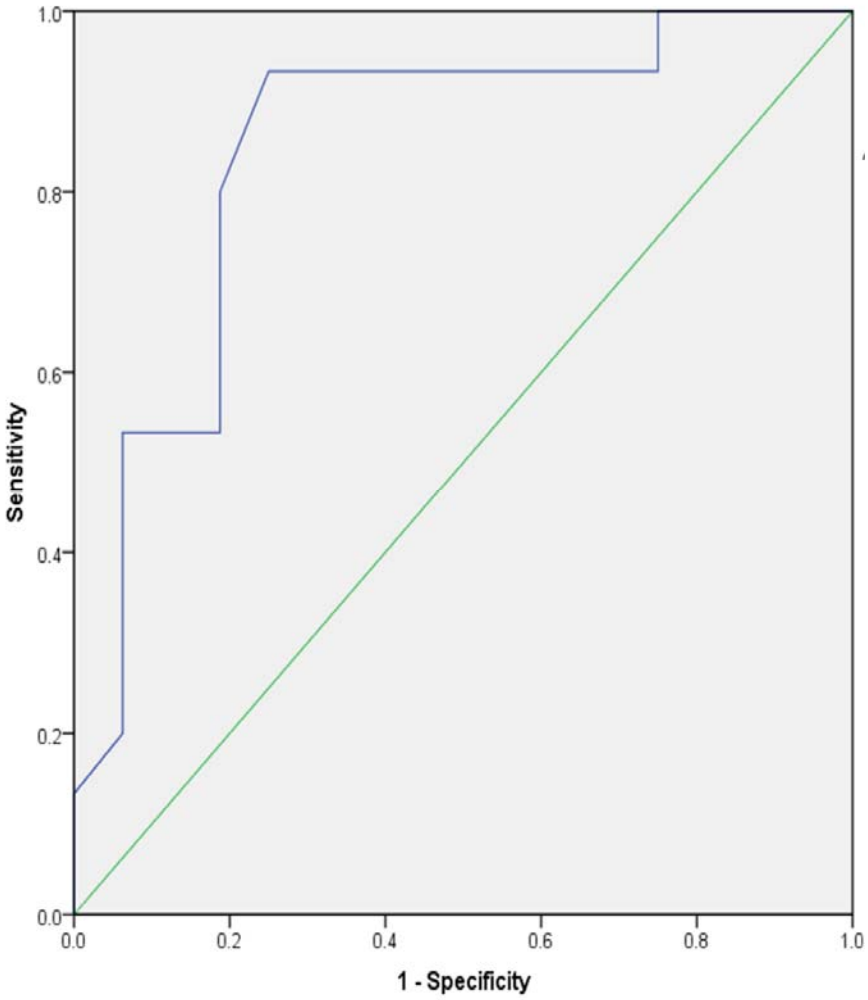

Fig. 1. Receiver Operator Curve showing the predictive ability of the multivariate logistic regression model (expressed as Cstatistics)

Table 1. Characteristics and Predictors for Neurosonogram Abnormalities in Infants Born to Rh-Immunized Women

Parameters	*Total Cohort (n = 71)*	*Comparison between Infants with and without IVH/PVL (Univariate analysis)*				*Multivariate analysis*	
		Neurosonogram abnormalities (n = 27)	*No Abnormalities (n = 44)*	*P value*	*Odds Ratio (95% CI)*	*Adjusted Odds Ratio (95% CI)*	*P value*
Gestational age (weeks)[a]	36 (35, 37)	36 (33, 36)	36 (35, 37)	0.012	-	0.7 (0.50, 0.97)	0.035
Birth Weight (grams)[b]	2545 (540)	2356 (655)	2662 (413)	0.04	-		
Small for gestational age[c]	11 (15.5)	7 (25.2)	4 (9.1%)	0.09		10.6 (1.9, 58.7)	0.007
Female[c]	30 (42.3)	12 (44.4)	18 (40.9%)	0.8	0.9 (0.3, 2.3)		
Intrauterine Characteristics							
Received IUT[c]	31 (43.7)	15 (55.6)	16 (36.4)	0.1	2.2 (0.8, 5.8)	3.3 (0.91, 11.9)	0.069
Received ≥ IUTs[c]	13 (18.3)	9 (33.3)	4 (9.1)	0.01	5 (1.4, 18.4)		
Number of IUT/ infant[a]	*n* = 31 0 (0, 2)	*n* = 15 1 (0, 3)	*n* = 16 0 (0, 1)	0.076	-		
Gestational age at first IUT (weeks)[b]	27.7 (4.3)	26 (4)	29.3 (4.1)	0.034	-		
Severe fetal anemia (Hct < 21%)[c]	22 (31)	12 (44.4)	10 (22.7)	0.055	2.7 (0.97, 7.7)		
Fetal hydrops[c]	7 (9.9)	4 (14.8)	3 (6.8)	0.4	2.4 (0.5, 11.6)		
Neonatal Characteristics							
Perinatal asphyxia[c]	10 (14.1)	7 (25.69)	3 (6.8)	0.036	4.8 (1.1, 20.5)	1.6 (0.3, 10.3)	0.6
Received DVET[c]	17 (23.9)	7 (25.9)	10 (22.7)	0.8	1.2 (0.4, 3.6)		
Neonatal Sepsis (Probable or Definite)[c]	28 (39.4) Proven (*n* = 2)	17 (63)	11 (25)	0.001	5.1 (1.8, 14.4)	4.5 (1.1, 18.4)	0.035
Hypoglycemia[c]	6 (8.5)	5 (18.5)	1 (2.3)	0.027	9.8 (1.1, 88.9)	2.4 (0.2, 30.2)	0.5
Required NICU admission[c]	19 (26.8)	14 (51.9)	5 (11.4)	< 0.001	-		
Duration of hospital stay (d)[a]	9 (7, 12)	11.5 (8, 19.8)	8 (6, 10)	< 0.001	-		
Death before discharge[c]	2 (2.8)	2	0	0.1	-		

Values expressed as [a]median (IQR), [b]mean (SD) or [c]n (%)

CI Confidence interval, DVET Double volume exchange transfusion, IUT Intrauterine transfusion, IVH Intraventricular hemorrhage, NICU Neonatal intensive care unit, OR Odds ratio, PVL Periventricular leukomalacia, SD Standard deviation