

Comparing Neurodevelopmental Outcome among Neonates with Jaundice Due to ABO and Rh Incompatibility

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ABSTRACT

Objectives: Evaluating risk factors is essential in the context of hyperbilirubinemia and its clinical outcomes, particularly when attributed to its primary causes: maternal-fetal ABO and Rh incompatibility. This study aims to determine the prevalence rate of predisposing factors and complications of hyperbilirubinemia in newborns with ABO blood group incompatibility, compared to those with Rh incompatibility.

Materials & Methods: This is a descriptive-analytical study. The newborns with jaundice who were referred to Ghaem Teaching Hospital affiliated to the Mashhad University of Medical Sciences, Mashhad, Iran, from 2017 to 2019, divided into two groups of ABO incompatibility (n = 83) and Rh incompatibility (n = 81). The neurodevelopmental assessment was performed by Denver Developmental Screening Test II (DDST-II). Following that, a comparison was made between these two groups.

Results: The mean age of the ABO incompatibility group was 5.84 days and the Rh incompatibility group was 4.91 days at admission time. The mean values of bilirubin level obtained at 28.26 ± 5.9 and 30.17 ± 8.19 in newborns with ABO and Rh incompatibility ($P = 0.089$). No significant difference was observed between the two groups regarding age, Apgar score, bilirubin level ($P > 0.05$), delivery type ($\chi^2 = 1.56$; $P = 0.21$), and gender ($\chi^2 = 0.403$; $P = 0.52$). Among the study population, 45.8% and 89.1% of the infants with Rh incompatibility and with ABO incompatibility had a near-normal neurological development in the first three years of life. A significant difference was found between the two groups regarding developmental status ($\chi^2 = 25.13$; $P < 0.05$).

Conclusion: Despite similar bilirubin levels and treatment approaches, neonates with Rh incompatibility experienced significantly higher rates of developmental delay compared to those with ABO incompatibility. By age three, moderate to severe delays were more frequent in the Rh group, underscoring the greater neurodevelopmental risk associated with Rh-mediated hemolysis.

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Introduction

The prevalence rates of jaundice are 60% and 80% among term and preterm infants in the first week of life. The rate of newborns with jaundice who require treatment (hyperbilirubinemia higher than 95% of

percentile) estimated between 8% and 11% [1, 2]. Although hyperbilirubinemia is benign in the majority of neonates, it may lead to cognitive impairment and developmental delay in some cases [3]. The most crucial cause of hyperbilirubinemia in neonates is

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hemolysis. Rapid accumulation of bilirubin may occur due to prematurity, infection, or certain blood disorders. ABO and Rh incompatibility are two main causes of hyperbilirubinemia. Accordingly, ABO incompatibility developed to hyperbilirubinemia in 15%-20% of all pregnancies [4-6]. The first-day bilirubin measurement is critical since it can predict the subsequent development of remarkable hyperbilirubinemia in healthy term neonates. About 10% of newborns with ABO incompatibility develop a hemolytic disease [7].

Considering that maternal-fetal ABO incompatibility can cause severe neonatal jaundice, and in some cases, may lead to cognitive impairment and developmental delay, such as kernicterus, it is of utmost importance to assess the potential risk factors for hyperbilirubinemia. Rh incompatibility is another main cause of hyperbilirubinemia in newborns. Although the frequency of hyperbilirubinemia due to ABO incompatibility is more common than Rh incompatibility, seemingly, Rh incompatibility is associated with more severe hyperbilirubinemia.

Several research efforts have focused on determining the outlook for patients with hemolytic jaundice [4, 8, 9]. However, very few studies have examined the differences between ABO and Rh incompatibility and how each affects hyperbilirubinemia in neonates. Furthermore, no studies compared the prognosis of ABO incompatibility and Rh in similar bilirubin levels. This study aimed to compare the neurodevelopmental outcomes of newborns with hyperbilirubinemia due to ABO incompatibility versus with those of Rh incompatibility, and to explore associated perinatal factors and complications.

Materials & Methods

Study design

This descriptive-analytical study was conducted on 164 patients with neonatal jaundice (83 patients with ABO incompatibility and 81 with Rh incompatibility) referred to Ghaem Teaching Hospital, affiliated to the Mashhad University of Medical Sciences, Mashhad, Iran, from 2017 to 2019.

Inclusion and exclusion criteria

The inclusion criteria were an age between 1 and 30 days, the presence of a yellowish discoloration of the sclera and skin indicative of clinical jaundice, and evidence of ABO or Rh incompatibility. On the other hand, the exclusion criteria were the neonates with cephalohematoma, infection, polycythemia, G6PD, congenital anomaly, and the concurrence of Rh or ABO

incompatibility, as well as the infants of diabetic mothers, and those with severe weight loss, along with refusing of the treatment or blood exchange transfusion.

Data collection

This study was conducted between 2017 to 2019 on the newborns younger than 30 days with jaundice admitted to the Neonatal Intensive Care Unit and Pediatric Emergency Department. The infants with more extent of mid-abdomen jaundice and normal physical examination were subjected to laboratory tests. Newborn's information, i.e., age, weight, birth weight, parity, Apgar score, bilirubin level, developmental status, and treatment approaches and maternal information, i.e., age and type of delivery were recorded in a researcher-made checklist by neonatal fellowship and neonatologist in this study. Rh incompatibility diagnosis was made when mother's Rh was negative, and neonate's Rh was positive plus positive direct Coombs test or decreased hemoglobin. ABO incompatibility was diagnosed when the mother's blood group was O+ and neonate's was A or B in addition to the presence of at least two of these conditions: 1) First-day jaundice, 2) The Positive direct Coombs test, 3) Microspherocytosis in peripheral blood smear, and 4) The positive indirect Coombs test. Newborns with jaundice were included and categorized into two groups of ABO incompatibility (n = 83) and Rh incompatibility (n = 81), and then compared with each other.

Neurodevelopmental assessment

The neurodevelopmental status of infants was assessed at 6, 12, 18, 24, 30, and 36 months using the Denver Developmental Screening Test II (DDST-II). The DDST-II is a standardized, internationally validated tool used to evaluate the developmental progress of children from birth to six years of age. It examines four key developmental domains: Personal-social, fine motor-adaptive, language, and gross motor skills [10]. In this study, all assessments were conducted by trained pediatricians and neonatal specialists who were certified in administering the DDST-II. An infant was considered developmentally delayed if a failure in any of the tested domains existed. Based on the number of affected domains, delays were classified as mild (one domain), moderate (two domains), or severe (three or more domains). Children were then grouped as having either normal development or developmental delay at age three, based on these classifications. Following that, the bilirubin levels of these infants compared to the admission time.

Ethical considerations

The proposal of the current study approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.MEDICAL.REC.1397.538). Regarding the ethical considerations, the information coded and then recorded in the checklists to assure confidentiality.

Statistical analysis

The data were analyzed using IBM SPSS software (version 23) through the Mann Whitney test, independent t-test, and Pearson chi-square test. The qualitative and quantitative variables represented as mean $\pm$ SD, as well as frequency and percentage. Furthermore, the normality of data assessed by the Kolmogorov-Smirnov test. A p-value less than 0.05 considered statistically significant.

Results

Out of 978 patients admitted to the hospital with jaundice, 202 and 86 infants were evaluated for ABO and Rh incompatibilities, respectively. Of the neonates with suspected ABO incompatibility, 119 were

excluded from the study due to the following reasons: G6PD deficiency (n = 33), concurrent Rh and ABO incompatibility (n = 21), prophylactic phototherapy without initial bilirubin measurement (n = 16), treatment refusal (n = 9), maternal diabetes (n = 9), severe weight loss (n = 8), hematoma (n = 7), infection (n = 5), polycythemia (n = 3), Down syndrome (n = 1), and parental refusal of blood transfusion (n = 7). Considering 16 infants received prophylaxis phototherapy, the mean bilirubin in the ABO incompatibility estimated at 24.33, significantly lower than that in the Rh incompatibility group. However, since this study aimed to compare the prognosis of both groups with the similar level of bilirubin, these 16 infants were excluded from this study. Finally, 83 neonates were included in this group. In addition, out of the Rh incompatible infants, two cases were excluded due to dissatisfaction with exchange transfusion. Following that, infection (n = 1), G6PD deficiency (n = 1), and cephalohematoma (n = 1) were the other reasons for the exclusion. Finally, 81 neonates with Rh incompatibility were investigated.

Table 1. Comparison of demographic and clinical characteristics between neonates with ABO and RH incompatibility

Variables	ABO incompatibility		Rh incompatibility		P-value
	Mean	SD	Mean	SD	
Age (day)	5.84	3.67	4.91	2.74	0.059
Weight at admission (gr)	3026.62	592.72	2988.98	404.08	0.475
Birth weight (gr)	3033.41	454.12	2876.71	472.90	0.107
Apgar score	9.71	0.13	10	0	0.552
Parity	1.63	0.89	2.38	1.38	0.005
Maternal age (year)	25.96	5.31	29.77	5.78	0.009
Bilirubin level (mg/dL)	28.26	5.96	30.17	8.19	0.089

SD: standard deviation.

Notably, 68.3% (n=112) of the neonates were followed up (64 neonates with ABO incompatibility and 48 neonates with Rh incompatibility). Table 1 tabulates the results of the comparison between Rh and ABO incompatibility regarding demographic characteristics. Based on the obtained results, the mean values of bilirubin level were obtained at 28.26 ± 5.9 and 30.17 ± 8.19 in newborns with ABO and Rh incompatibility, respectively ($P = 0.089$). Moreover, a significant difference was observed between the two groups regarding parity and maternal age ($P < 0.05$). However, no significant difference was observed between the two groups regarding age, Apgar score, and bilirubin level ($P > 0.05$).

The results of the comparison between Rh and ABO incompatibility regarding developmental status, delivery type, gender, and treatment approaches are presented in Table 2. The results revealed a significant

difference between the two groups regarding developmental status ($\chi^2 = 25.13$; $P < 0.05$). During follow-up, 71.9% of the neonates with ABO incompatibility had normal neuropsychiatric development, and 28.2% of the cases had developmental delay, 18.8%, 4.7%, and 4.7% of which were mild, moderate, and severe, respectively.

If mild cases ignored, 90.7% of the infants with ABO incompatibility had near-normal life in the first three years of life, and 39.6% of the neonates with Rh incompatibility had normal development. Moreover, 60.4% of the neonates had developmental delay, 8.3%, 22.9%, and 29.2% of which were mild, moderate, and severe, respectively. If mild cases ignored, 47.9% of the infants with Rh incompatibility had a near-normal life in the first three years of life.

Furthermore, no significant difference was found between the two groups of ABO and Rh

incompatibility in terms of delivery type ($\chi^2 = 1.56$; $P = 0.21$) and gender ($\chi^2 = 0.403$; $P = 0.52$).

Moreover, no significant difference was found between the two groups in terms of treatment approaches, including phototherapy alone, phototherapy plus

exchange transfusion, phototherapy plus intravenous immunoglobulin G (IVIG) therapy, and exchange transfusion plus IVIG therapy ($\chi^2 = 3.17$; $P = 0.36$).

Table 2. Comparison of developmental outcomes, delivery mode, gender, and treatment approaches between neonates with ABO and Rh incompatibility

Variables		ABO incompatibility		Rh incompatibility		Total		χ^2	P-value
		No	%	No	%	No	%		
Developmental status	Normal	46	71.9	19	39.6	65	58	25.13	<0.05
	Mean bilirubin = 23.97 mg/dL								
	Mild developmental delay	12	18.8	4	8.3	16	14.3		
	Mean bilirubin = 31.97 mg/dL								
	Moderate developmental delay	3	4.7	11	22.9	14	12.5		
	Mean bilirubin = 34.72 mg/dL								
	Severe developmental delay	3	4.7	14	29.2	17	15.2		
	Mean bilirubin = 38.29 mg/dL								
Mode of delivery	Vaginal delivery	39	53.4	44	63.8	83	58.5	1.56	0.21
	Cesarean section	34	46.6	25	36.2	59	41.5		
Gender	Male	37	46.8	42	51.9	79	49.4	0.4	0.52
	Female	42	53.2	39	48.1	81	50.6		
Treatment	Phototherapy	9	11.1	5	6.2	14	8.6	3.17	0.36
	Phototherapy and exchange transfusion	27	88.9	74	91.4	146	90.1		
	IVIG and phototherapy	0	0	1	1.2	1	0.6		
	IVIG and exchange transfusion	0	0	1	1.2	1	0.6		

In both Rh and ABO incompatibilities, the rate of developmental delay worsened with increasing bilirubin levels. Accordingly, the mean values of bilirubin were 23.97, 31.97, 34.72, and 38.29 mg/dL in neonates with good prognosis, as well as mild,

moderate, and severe developmental delays, respectively.

Figure 1 shows comparison between Rh and ABO incompatibility groups in terms of developmental status.

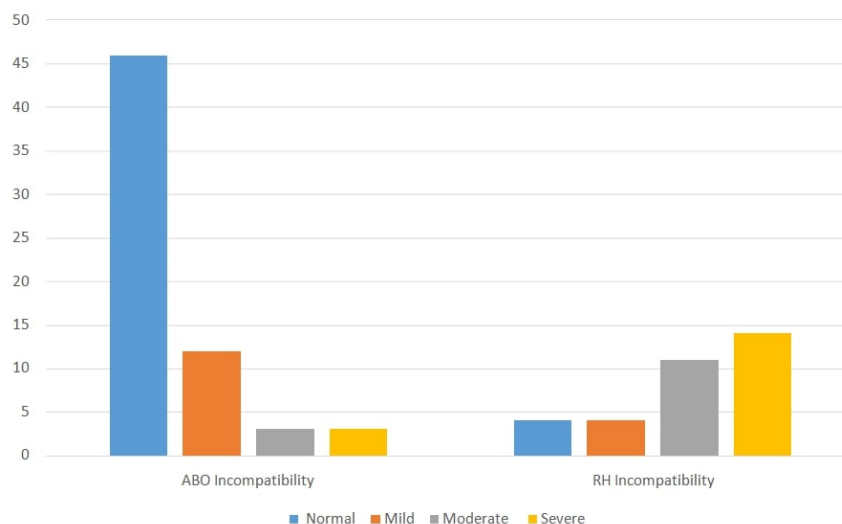


Figure 1. Comparison between Rh and ABO incompatibility groups in terms of developmental status. Distribution of neurodevelopmental outcomes (normal, mild, moderate, and severe developmental delay) in neonates with ABO and Rh incompatibility. Rh-incompatible infants demonstrated a higher proportion of moderate and severe developmental delays compared to those with ABO incompatibility.

Discussion

The referral age of the neonates with Rh and ABO incompatibility was 5 to 6 days, which was too late for treatment and might have been one of the factors in the

poor prognosis of these infants [22, 23]. Furthermore, the number of pregnancies and the age of mothers of neonates experiencing Rh incompatibility were notably higher compared to mothers of infants with ABO

incompatibility. This observation appears reasonable, given that Rh incompatibility tends to increase with a higher number of pregnancies. This study's results showed that similar bilirubin level in neonates with Rh incompatibility led to worse prognosis, compared to those with ABO incompatibility. Accordingly, the probability of the developmental delay at the age of three in the Rh incompatibility infants is more than five times, compared to neonates with ABO incompatibility with similar bilirubin level.

More than two-thirds of the neonates with jaundice and ABO incompatibility who received appropriate treatment will have a normal developmental life. However, despite proper treatment, a degree of developmental delay was observed in more than 60% of the neonates with Rh incompatibility by age three.

The prognosis of neonatal jaundice due to Rh and ABO incompatibilities worsened with increasing the severity of hyperbilirubinemia. This, in turn, further worsened the prognosis in cases of Rh incompatibility.

The study results showed that the neonates with ABO incompatibility had higher current weight, and birth weight, compared to those with Rh incompatibility. Moreover, the parity and maternal age were lower in neonates with ABO incompatibility, compared to newborns with Rh incompatibility. On the other hand, no difference reported between the two groups of neonates regarding Apgar score and bilirubin level. Moreover, the developmental status of the neonates with ABO incompatibility was better than those with Rh incompatibility. Notably, no difference was found between the neonates with Rh/ABO incompatibility in terms of gender, delivery type, and treatment approaches.

However, the developmental status of the neonates with Rh/ABO incompatibility born by vaginal delivery was better than those delivered by cesarean section. Moreover, the developmental delay was reported more among male, compared to female newborns.

In general, no evidence of the lower birth weight of newborns with ABO incompatibility is available. According to a study conducted by Akgül et al., the mean birth weight of newborns with ABO incompatibility was 3,142 grams, consistent with the findings in the present study (3,033 gram) [11]. Sarici et al. showed no difference in birth weight between neonates with remarkable hyperbilirubinemia due to ABO incompatibility and those without remarkable hyperbilirubinemia [12]. ABO incompatibility does not seem to be related to low birth weight in newborns with jaundice. In a study performed by Weng et al., no significant difference was observed between the frequency of male and female neonates in terms of blood group incompatibility. However, the number of

male newborns with Rh incompatibility was higher than that of those with ABO incompatibility [13]. Nonetheless, no significant difference was found between ABO and Rh incompatibility regarding gender and delivery type. However, Weng et al. reported that neonates delivered by cesarean section tended to have Rh incompatibility [13]. These differences may be due to the influence of intervention variables. Very few studies are available comparing ABO and Rh incompatibility in terms of clinical characteristics. Therefore, future studies are recommended to be conducted in this regard.

The ABO and Rh incompatibility, as well as glucose-6-phosphate dehydrogenase (G6PD) deficiency, are the main causes of hyperbilirubinemia. However, this disorder may be caused by other conditions [14]. The ABO incompatibility develop hyperbilirubinemia in one-third of neonates [4]. Kainiet al., assessed 293 neonates with hyperbilirubinemia whose common causes of jaundice were ABO (11%), and Rh isoimmunization (7.4%) [15]. The ABO incompatibility (17%), Rh isoimmunization (7%), G6PD deficiency (6%), and minor blood group incompatibility (2%) were responsible for hyperbilirubinemia in descending order [16]. In the present study, 50.6% and 49.4% of the neonates had ABO and Rh incompatibility.

According to Boskabadi et al.'s study, the hemolytic disease was the most common pathologic cause of hyperbilirubinemia in newborns; moreover, 40% and 18% of the newborns suffered from ABO and Rh incompatibility, respectively. Jaundice had appeared earlier in hemolytic cases, compared to idiopathic patients. They also showed the earlier appearance of jaundice in cases with Rh incompatibility, ABO incompatibility, and G6PD deficiency in descending order [4].

Akgül et al. reported that blood type did not affect the severity of hemolytic jaundice in ABO incompatibility [11]. In addition, no difference was found between ABO and Rh incompatibility regarding bilirubin levels. However, another similar study reported more severe hyperbilirubinemia among newborns with Rh incompatibility, compared to those with ABO incompatibility. In a study conducted by Weng et al., The mean serum bilirubin level was calculated at 25.8 in ABO incompatibility [13]. In the present study, the mean values of serum bilirubin were determined at 28.2 and 30.17 in ABO and Rh incompatibility groups. However, no difference was observed among patients with various blood groups. Akgül et al. found no significant differences between newborns with A and B blood group incompatibility, in terms of hematological parameters, such as initial

and final indirect bilirubin levels. Moreover, they concluded that blood type had no effects on the severity of hemolytic jaundice in ABO incompatibility [11].

The more severity of the disease reported in cases with hemolytic hyperbilirubinemia, in which clinical jaundice appears earlier. Therefore, earlier diagnosis of jaundice is more plausible in cases with Rh incompatibility due to the higher level of serum bilirubin in the Rh disease [17]. High bilirubin levels lead to acute bilirubin encephalopathy and brain damage in the short and long terms, respectively. High bilirubin levels could also damage the developing central nervous system [18]. The occurrence of jaundice within two days of life is considered a significant risk factor for abnormal developmental quotient [19].

A better developmental status was found among neonates with ABO incompatibility, compared to those with Rh incompatibility. Similar to the present study's results, Rasul et al. showed a higher rate of Kernicterus among neonates with Rh incompatibility, compared to those with ABO incompatibility [20]. Based on the results of a study conducted by Weng et al., blood group incompatibility in neonates leads to an early onset of hyperbilirubinemia. Moreover, they demonstrated that the newborns with blood group incompatibility at a peak total serum bilirubin level higher than 20 mg/dL were at higher risk of developing Kernicterus, compared to those without hemolysis [13].

Early detection of hyperbilirubinemia is critical in this regard because it enables prompt initiation of treatment, helps prevent potential complications associated with phototherapy, and reduces the need for exchange transfusion and/or IVIG. However, the diagnosis of hyperbilirubinemia complicated due to the limited predictive value of the current screening measures [14]. Clinical examination, direct measurement of serum bilirubin levels, and direct Coombs test are the existing detection methods for hyperbilirubinemia [21]. In this regard, no significant difference was observed between Rh and ABO incompatibility in terms of treatment approaches, including IVIG, phototherapy, and exchange transfusion. Weng et al. claimed that exchange transfusions commonly used among neonates with Rh incompatibility [13]. These differences may attribute to the influence of intervention variables or sample differences. Since a dearth of research exists, comparing the treatment approaches for hyperbilirubinemia, future studies are recommended to be conducted in this regard.

Limitations

To the best of our knowledge, this study is the first to compare ABO and Rh incompatibility in Iranian neonates with hyperbilirubinemia at similar bilirubin levels upon admission. However, several limitations should be noted. First, due to the retrospective design, the findings may be subject to selection bias and have limited generalizability. Second, while this study used the DDST-II to assess neurodevelopmental outcomes, we reported only the overall developmental status without analyzing specific domains, limiting the granularity of these findings. Finally, given the limited number of similar studies, further prospective research with detailed developmental assessments is recommended to validate and expand upon the obtained results.

In Conclusion

Despite similar bilirubin levels and treatment approaches, this study demonstrated a significant difference in neurodevelopmental outcomes between neonates with ABO and Rh incompatibilities. While 90.7% of infants with ABO incompatibility experienced normal or near-normal development by age three, only 47.9% of those with Rh incompatibility achieved similar outcomes. Notably, moderate to severe developmental delays were markedly more common in the Rh incompatibility group. These findings suggest that Rh incompatibility may pose a substantially greater risk to long-term neurodevelopmental health than ABO incompatibility, even at comparable bilirubin levels. Therefore, early identification, close monitoring, and aggressive management of Rh-incompatible neonates are crucial to reducing the risk of adverse outcomes.

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The proposal of the current study approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.MEDICAL.REC.1397.538). Regarding the Ethical Considerations, the information coded and then recorded in the checklists to assure confidentiality.

Authors' Contributions

The authors confirm contribution to the paper as follows: Study conception and design: Hassan Boskabadi and Shima Imannezhad; data collection: Fatemeh Bagheri and Elnaz Farajirad; analysis and interpretation of results: Hassan Boskabadi, Shima Imannezhad, Farima Farsi, and Elnaz Farajirad; draft manuscript preparation: Hassan Boskabadi, Shima Imannezhad, and Farima Farsi. All authors reviewed

the results and approved the final version of the manuscript.

Conflict of Interest

The authors declared no conflict of interest.

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