Original Article

doi:10.31674/mjn.2026.v18i03.013

MJN

Maternal–Neonatal ABO and Rh Incompatibility and Total Serum Bilirubin Levels among Jaundiced Neonates: An Analytical Cross-Sectional Study in Public Hospitals in Karbala, Iraq

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ABSTRACT

Background: Neonatal jaundice is a common neonatal condition characterized by elevated bilirubin levels. Maternal-neonatal ABO and Rh blood group incompatibility may contribute to hemolysis, but its frequency and relationship with bilirubin severity among jaundiced neonates vary across populations. This study aimed to determine the prevalence of maternal-neonatal ABO and Rh incompatibility among jaundiced neonates and assess its association with Total Serum Bilirubin (TSB) levels among neonates admitted to public hospitals in Karbala, Iraq. **Methods:** An analytical cross-sectional study was conducted among 386 jaundiced neonates admitted to Karbala Children's Teaching Hospital and Al-Hindiya Teaching Hospital in Karbala Governorate, Iraq, between late October 2024 and April 2025. Data were obtained through maternal interviews and hospital records. Blood group and Rh factor were determined for each mother-neonate pair, and TSB was measured for the neonates. Descriptive statistics summarized the study variables. Associations between maternal–neonatal ABO and Rh incompatibility and categorized total serum bilirubin (TSB) levels were assessed using Pearson's chi-square test or Fisher's exact test, as appropriate. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength and precision of these associations. **Results:** Among the 386 jaundiced neonates, 92.2% had TSB levels <18 mg/dL and 7.8% had levels ≥18 mg/dL; the mean TSB was 14.03 ± 3.05 mg/dL. Neonatal blood group A was the most frequent (39.1%), and 95.1% of neonates were Rh-positive. Maternal–neonatal ABO incompatibility was identified in 29.0% of pairs, whereas Rh incompatibility was identified in 7.8%. Neither ABO incompatibility (OR = 1.05, 95% CI: 0.47–2.38; $P = 0.902$) nor Rh incompatibility (OR = 1.35, 95% CI: 0.39–4.75; $P = 0.718$) showed a statistically significant association with categorized TSB levels. **Conclusion:** No statistically significant association was detected between maternal–neonatal ABO or Rh incompatibility and categorized TSB levels. However, these findings do not exclude a possible association, given the limited statistical precision and potential confounding. Further research is needed to clarify these relationships.

Keywords: ABO Incompatibility; Neonatal Jaundice; Neonates; Rh Incompatibility; Total Serum Bilirubin

Introduction

Neonatal jaundice, also known as neonatal hyperbilirubinemia or icterus, is characterized by elevated bilirubin concentrations and yellow discoloration of the skin and sclera resulting from bilirubin accumulation in tissues (Bahr & Gautham, 2026). It affects approximately 60% of term and 80% of preterm neonates during the first week of life (Murekatete *et al.,* 2020). Globally, an estimated 1.1 million newborns develop severe neonatal hyperbilirubinemia annually, with a disproportionate burden in sub-Saharan Africa and South Asia (Olusanya *et al.,* 2015). Neonates are particularly susceptible to hyperbilirubinemia because of increased bilirubin production associated with shorter red blood cell survival, immature hepatic bilirubin conjugation,

Received: April 21, 2026 Received in revised form: October 8, 2026 Accepted: October 20, 2026

and increased enterohepatic circulation (Bahr & Gautham, 2026).

Physiological jaundice typically appears after the first 24 hours of life, peaks between the third and fifth days, and gradually resolves as bilirubin metabolism matures. In contrast, pathological jaundice may develop within the first 24 hours or exhibit an abnormal pattern of bilirubin elevation, potentially resulting from hemolytic disorders, infections, or other underlying conditions (Donkor *et al.,* 2025). Severe unconjugated hyperbilirubinemia can lead to bilirubin-induced neurological dysfunction, including kernicterus, if bilirubin accumulates in the central nervous system (Tessema *et al.,* 2024). Therefore, timely assessment of bilirubin concentrations, identification of relevant clinical risk factors, and appropriate interventions, including phototherapy when clinically indicated, are essential components of neonatal jaundice management (Kemper *et al.,* 2022). Reviews of neonatal hyperbilirubinemia describe the diagnostic and treatment considerations associated with bilirubin elevation (Nawaz *et al.,* 2021). Earlier research on bilirubin measurement has also informed approaches to identifying neonates at risk of significant hyperbilirubinemia (Bhutani *et al.,* 2000). Phototherapy remains an established treatment for neonatal hyperbilirubinemia, with its development and clinical application reviewed in the literature (Maisels & McDonagh, 2008; Hansen *et al.,* 2020).

In Iraq, previous studies have investigated neonatal jaundice and its associated maternal and neonatal factors, including hemolytic disorders, ABO and Rh incompatibility, and glucose-6-phosphate dehydrogenase deficiency (Al-Jiboury *et al.,* 2023). Maternal–neonatal ABO incompatibility most commonly occurs when a mother with blood group O delivers a neonate with blood group A or B, potentially resulting in immune-mediated hemolysis through the transplacental transfer of maternal IgG antibodies (Mwenesi, 2023). However, blood-group incompatibility does not necessarily indicate clinically significant hemolysis, and evidence from studies of ABO-incompatible neonates highlights the importance of distinguishing blood-group mismatch from laboratory findings such as direct antiglobulin test results (Ercin *et al.,* 2024). Hospital-based studies in other settings have also examined maternal and neonatal factors associated with jaundice and indirect hyperbilirubinemia (Belay *et al.,* 2023; Isa *et al.,* 2022). Despite existing research on neonatal jaundice in Iraq, evidence regarding the prevalence of maternal–neonatal ABO and Rh incompatibility and their association with bilirubin levels among jaundiced neonates remains limited, particularly in Karbala Governorate. Therefore, this study aimed to determine the prevalence of maternal–neonatal ABO and Rh incompatibility among jaundiced neonates admitted to public hospitals in Karbala Governorate, Iraq, and to assess the association between blood-group incompatibility and total serum bilirubin levels. A recent review of hemolytic disease of the fetus and newborn describes maternal red-cell antibodies as an important mechanism underlying fetal and neonatal hemolysis (Malik *et al.*, 2025). Guideline comparisons also emphasize prevention of maternal RhD alloimmunization through appropriate anti-D prophylaxis (Boureka *et al*., 2024).

METHODOLOGY

Study Design and Setting

An analytical cross-sectional study was conducted at Karbala Children's Teaching Hospital and Al-Hindiya Teaching Hospital in Karbala Governorate, Iraq, to determine the prevalence of maternal–neonatal ABO and Rh incompatibility and assess its association with total serum bilirubin (TSB) levels among hospitalized jaundiced neonates. Participant recruitment and data collection began after 22 October 2024 and continued until April 2025, following ethical approval and administrative permission. The study design and reporting were guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations (Von Elm *et al.,* 2007).

Sampling Technique and Sample Size

A non-probability convenience sampling technique was employed to recruit neonates diagnosed with jaundice who were admitted to Karbala Children's Teaching Hospital and Al-Hindiya Teaching Hospital during the study period. Initially, 408 neonates were recruited. Of these, 22 (5.4%) were excluded because of incomplete questionnaire data, leaving 386 neonates (94.6%) in the final analysis. The participant recruitment and inclusion process is illustrated in Figure 1.

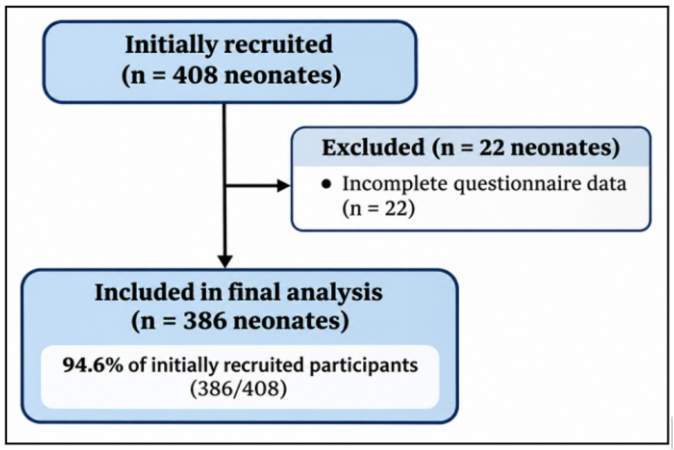


Figure 1: Participant Recruitment and Inclusion Flow

Inclusion Criteria

Neonates aged 1–28 days who were admitted to the participating hospitals with a clinical diagnosis of neonatal jaundice were eligible for inclusion. Total serum bilirubin (TSB) measurements were obtained from hospital laboratory records.

Exclusion Criteria

Neonates residing outside Karbala Governorate, those receiving care exclusively at private hospitals or outpatient clinics, and hospitalized neonates with incomplete questionnaire information were excluded from the study.

Data Collection

Data were collected using an interviewer-administered questionnaire and hospital laboratory records. The questionnaire included neonatal and parental demographic and clinical characteristics. Of the 386 neonates included in the final analysis, 218 (56.5%) were recruited from Karbala Children's Teaching Hospital and 168 (43.5%) from Al-Hindiya Teaching Hospital. Maternal and neonatal ABO blood groups, Rh status, and neonatal total serum bilirubin (TSB) concentrations were obtained from hospital laboratory records. The TSB measurement obtained at hospital admission was used for statistical analysis. Paternal blood group and Rh status were recorded as background characteristics based on information provided during maternal interviews.

Classification of ABO and Rh incompatibility

Maternal–neonatal ABO incompatibility was operationally defined as a mother with blood group O and a neonate with blood group A or B. All other observed maternal–neonatal ABO combinations were classified as compatible. Rh incompatibility was defined as an Rh-negative mother with an Rh-positive neonate, whereas all other Rh combinations were classified as compatible.

TSB Classification

For statistical analysis, total serum bilirubin (TSB) concentrations were categorized into two groups: <18 mg/dL and ≥18 mg/dL. This operational threshold was used to compare bilirubin levels between maternal–neonatal ABO- and Rh-compatible and incompatible groups.

Ethical Consideration

This study received ethical approval from the Presidency of Al-Furat Al-Awsat Technical University, Iraq, with reference number 4835 on 20 October 2024. Administrative permission to conduct the research and collect data at the participating public hospitals was granted by the Karbala Health Directorate through the Training and Human Development Center/Research and Knowledge Management Unit, with reference

number 3685 on 22 October 2024. Verbal informed consent was obtained from the mothers before participation.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 29. Categorical variables were summarized as frequencies and percentages, while continuous variables were expressed as means and standard deviations. The prevalence of maternal–neonatal ABO and Rh incompatibility was calculated among the 386 included neonates. Associations between ABO and Rh incompatibility and categorized TSB levels (<18 mg/dL versus ≥18 mg/dL) were assessed using Pearson's chi-square test or Fisher's exact test, as appropriate. Crude odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated to estimate the strength and precision of these associations. Although gestational age and birth weight were collected and summarized descriptively, they were not included as covariates in an adjusted statistical model. No multivariable adjustment was performed. Statistical significance was defined as $P < 0.05$.

RESULTS

Table 1 shows that most neonates were male (64.0%), with a male-to-female ratio of 1.8:1. Most neonates (61.0%) had normal birth weight (2.5–4.0 kg), with a mean birth weight of 2.65 ± 0.68 kg. Furthermore, 59.1% were admitted during the first week of life (1–7 days), with a mean age at admission of 8.33 ± 5.55 days. In terms of gestational age, 60.6% were full-term neonates (37–41 weeks).

Table 1: Distribution of Neonates According to Sex, Birth Weight, Age at Admission, and Gestational Age

Variable	Category	No. (%)
Sex	Male	247 (64.0)
	Female	139 (36.0)
Birth Weight (kg)	< 2.5 kg	143 (37.0)
	2.5–4.0 kg	235 (61.0)
	> 4.0 kg	8 (2.0)
Mean ± SD	**2.65 ± 0.68**	
Age at Admission (days)	1–7	228 (59.1)
	8–15	103 (26.7)
	16–21	35 (9.1)
	22–28	20 (5.2)
Mean ± SD	**8.33 ± 5.55**	
Gestational Age (weeks)	< 37 (Preterm)	150 (38.9)
	37–41 (Full-term)	234 (60.6)
	≥ 42 (Post-term)	2 (0.5)

Table 2 shows that most neonates (92.2%) had a TSB level <18 mg/dL, while 7.8% had TSB ≥18 mg/dL, with a mean TSB of 14.03 ± 3.05 mg/dL. Blood groups A (39.1%) and O (36.8%) were the most frequent neonatal ABO groups, and the majority of neonates (95.1%) were Rh-positive.

Table 2: Distribution of TSB Level, ABO Blood Group, and Rh Factor among Jaundiced Neonates (n = 386)

Variable	Category	No.	Percentage (%)
TSB level (mg/dL)	<18	356	92.2
	≥18	30	7.8
ABO blood group	A	151	39.1
	B	75	19.4
	AB	18	4.7
	O	142	36.8
Rh factor	Rh-positive	367	95.1
	Rh-negative	19	4.9

Table 3 presents parental blood group and Rh factor distributions. Blood group O was the most frequent group among fathers (53.6%) and mothers (57.0%). Most fathers (95.1%) and mothers (89.1%) were Rh-

positive. Paternal blood-group data were presented descriptively and were not used to define maternal–neonatal incompatibility or in the main association analyses.

Table 3: Distribution of Parental ABO Blood Groups and Rh Factors

Blood group & RH Factor	Father		Mother	
	No.	%	No.	%
A	90	23.3	94	24.4
B	46	11.9	41	10.6
AB	43	11.1	31	8.0
O	207	53.6	220	57.0
Total	**386**	**100**	**386**	**100**
Rh-positive	367	95.1	344	89.1
Rh-negative	19	4.9	42	10.9
Total	**386**	**100**	**386**	**100**

Table 4 presents the distribution of maternal–neonatal ABO and Rh compatibility among the 386 jaundiced neonates. ABO incompatibility was identified in 112 mother–neonate pairs (29.0%), whereas 274 pairs (71.0%) were classified as ABO-compatible. Rh incompatibility was identified in 30 pairs (7.8%), while 356 pairs (92.2%) were Rh-compatible.

Table 4: Prevalence of Maternal–Neonatal ABO and Rh Compatibility Among Jaundiced Neonates

Category	No.	%
ABO compatibility	274	71.0
ABO incompatibility	112	29.0
Rh compatibility	356	92.2
Rh incompatibility	30	7.8
Total	**386**	**100**

Table 5 shows no statistically significant association between maternal–neonatal ABO or Rh incompatibility and TSB level. TSB ≥18 mg/dL was observed in 8.0% of ABO-incompatible pairs compared with 7.7% of ABO-compatible pairs (OR = 1.05, 95% CI: 0.47–2.38; $P = 0.902$). Similarly, 10.0% of Rh-incompatible pairs had TSB ≥18 mg/dL compared with 7.6% of Rh-compatible pairs (OR = 1.35, 95% CI: 0.39–4.75; $P = 0.718$). These findings indicate that neither ABO nor Rh incompatibility was significantly associated with higher categorized TSB levels. Only 30 neonates had TSB ≥18 mg/dL, limiting the precision of these comparisons.

Table 5: Association of Maternal–Neonatal ABO and Rh Incompatibility with Total Serum Bilirubin Level

Blood-Group Compatibility	TSB <18 mg/dL, n (%)	TSB ≥18 mg/dL, n (%)	Crude OR (95% CI)	*P*-value
ABO compatible	253 (92.3)	21 (7.7)	Reference	**0.902**[a]
ABO incompatible	103 (92.0)	9 (8.0)	1.05 (0.47–2.38)	
Rh compatible	329 (92.4)	27 (7.6)	Reference	**0.718**[b]
Rh incompatible	27 (90.0)	3 (10.0)	1.35 (0.39–4.75)	

[a]Pearson's chi-square test; [b]Fisher's exact test. OR odds ratio; CI, confidence interval

DISCUSSION

This analytical cross-sectional study investigated maternal–neonatal ABO and Rh incompatibility and their associations with total serum bilirubin (TSB) levels among hospitalized jaundiced neonates in Karbala, Iraq. Previous studies from Iraq and Iran have described variations in bilirubin concentrations and the characteristics of neonates admitted with jaundice (Yahya & Alajeely, 2013; Saadat *et al.,* 2018). Such variations may reflect differences in study populations, neonatal characteristics, admission practices, and

clinical settings. The present findings contribute hospital-based evidence concerning blood-group incompatibility among jaundiced neonates in Karbala Governorate.

Maternal–neonatal ABO incompatibility was identified in 29.0% of the study population, whereas Rh incompatibility was identified in 7.8%. These frequencies are comparable to those reported by Bolat *et al.* (2010), who observed ABO and Rh incompatibility in 29.2% and 6.4% of neonates with indirect hyperbilirubinemia, respectively. In Pakistan, Irshad *et al.* (2011) reported ABO incompatibility in 22.5% and Rh incompatibility in 12.5% of 200 jaundiced neonates. Similarly, Sherani *et al.* (2026) reported ABO incompatibility in 33.0% and Rh incompatibility in 17.0% of 300 jaundiced neonates in Najaf, Iraq, with combined ABO/Rh incompatibility reported separately. Although the ABO incompatibility frequency in the present study was broadly consistent with these reports, differences in Rh incompatibility frequencies were more pronounced. These variations may be related to differences in maternal and neonatal blood-group distributions, participant selection, study settings, and operational definitions of incompatibility. Consequently, comparisons between studies should be interpreted with consideration of their methodological differences.

Neither maternal–neonatal ABO incompatibility (crude OR = 1.05, 95% CI: 0.47–2.38; $P = 0.902$) nor Rh incompatibility (crude OR = 1.35, 95% CI: 0.39–4.75; $P = 0.718$) demonstrated a statistically significant association with TSB concentrations categorized at 18 mg/dL. These findings indicate that the present analysis did not detect a significant difference in the odds of having TSB ≥18 mg/dL between compatible and incompatible mother–neonate pairs. However, the wide confidence intervals, particularly for Rh incompatibility, indicate considerable uncertainty regarding the magnitude and direction of the associations. Therefore, the findings should not be interpreted as evidence that blood-group incompatibility has no relationship with bilirubin concentrations. In a clinical pathway study of ABO-incompatible newborns with negative direct antiglobulin tests, phototherapy rates did not significantly increase after low-risk classification, illustrating that incompatibility alone does not determine treatment need (Gabbay *et al*., 2023).

The interpretation of these findings requires consideration of the distinction between maternal–neonatal blood-group incompatibility and immune-mediated hemolytic disease. Although ABO and Rh incompatibility can contribute to neonatal hyperbilirubinemia through immune-mediated hemolysis, blood-group mismatch alone does not establish that clinically significant hemolysis has occurred (Mwenesi, 2023). Previous research involving ABO-incompatible neonates has highlighted the importance of direct antiglobulin test findings when evaluating the risk of significant hyperbilirubinemia (Ercin *et al.,* 2024). Accordingly, the observed absence of a statistically significant association should be interpreted in light of the distinction between blood-group incompatibility and confirmed immune-mediated hemolysis. The clinical interpretation of bilirubin concentrations requires consideration of postnatal age, gestational age, and additional risk factors, particularly those associated with bilirubin neurotoxicity (Kemper *et al.,* 2022). Consequently, maternal–neonatal blood-group incompatibility should be interpreted alongside the broader clinical and laboratory assessment rather than as an isolated indicator of bilirubin severity. Research evaluating practice after the 2022 American Academy of Pediatrics guideline further illustrates the importance of considering contemporary bilirubin measurement and treatment recommendations (Sarathy *et al.,* 2024). A systematic review and meta-analysis reported limited sensitivity of the direct antiglobulin test for predicting phototherapy in neonates with ABO or Rh incompatibility, underscoring the need for broader clinical assessment (Kumar Krishnegowda *et al*., 2025).

Limitations

Several limitations should be considered when interpreting the findings. The hospital-based convenience sample, restricted to neonates with jaundice, limits generalizability and prevents assessment of maternal–neonatal blood-group incompatibility as a risk factor for the occurrence of neonatal jaundice. The use of a fixed, non-age-specific total serum bilirubin (TSB) threshold and the categorization of TSB rather than its analysis as a continuous variable may have obscured clinically relevant differences. The small number of

neonates with TSB ≥18 mg/dL, particularly among Rh-incompatible pairs, reduced the precision of the association estimates. Although gestational age and birth weight were collected and reported descriptively, neither was included in an adjusted statistical model, and the reported associations remained unadjusted. Other potentially relevant factors, including glucose-6-phosphate dehydrogenase deficiency, feeding practices, and treatment before admission, were not consistently documented or incorporated into the analysis. Incomplete laboratory information on hemolysis prevented confirmation of immune-mediated hemolytic disease, while uncertainty regarding treatment timing and possible differences in laboratory procedures between hospitals may have affected the comparability of bilirubin measurements. Finally, convenience sampling and the exclusion of participants with incomplete questionnaire data may have introduced selection bias.

Future Scope

Future research should employ prospective, multicenter designs with larger sample sizes and include non-jaundiced comparison groups when investigating risk factors for the occurrence of neonatal jaundice. Bilirubin concentrations should be analyzed as continuous outcomes alongside postnatal age- and gestational age-specific clinical thresholds. Incorporating standardized hemolysis markers, treatment histories, and relevant maternal and neonatal characteristics would support more informative adjusted analyses. Additional comparisons between participating hospitals may also help clarify the influence of differences in patient characteristics and clinical practices on the observed associations. A review of maternal alloimmunization further emphasizes that transplacental maternal antibodies may cause fetal or neonatal hemolysis and anemia, supporting the inclusion of antibody and hemolysis assessments in future studies (MacGregor, 2024).

CONCLUSION

This study provides hospital-based evidence on the frequency of maternal–neonatal ABO and Rh incompatibility among jaundiced neonates in Karbala, Iraq. The findings support interpreting maternal–neonatal blood-group compatibility as one component of neonatal jaundice assessment rather than as an independent indicator of bilirubin severity. Further prospective studies incorporating age-specific bilirubin assessment and laboratory confirmation of hemolysis are warranted to clarify the relationship between blood-group incompatibility and neonatal hyperbilirubinemia.

CRediT Authorship Contribution Statement

D. A. A.S.: Conceptualization, Methodology, Investigation, Data collection, Formal AAnalysis, Writing – original draft. **M.S.A.N.:** Supervision, Validation, Methodology review, Writing – review and editing.

AI Assistance Declaration

The authors utilized ChatGPT software to help with grammar and language editing while preparing this manuscript. The authors carefully reviewed and revised all created content, and they are solely responsible for the final manuscript's accuracy and integrity.

Conflict of Interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Prof. Dr. Oday Mitib Hadi, Head of the Department, for his general administrative support and encouragement. They also acknowledge the staff of the Community Health Technologies Department, Karbala Health Directorate, Al-Hindiya Teaching Hospital, and Karbala Children's Teaching Hospital for their technical assistance and cooperation during data collection. The authors are grateful to the participating neonates' mothers for their cooperation.

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