Prevalence and Comparative Clinical Profile of Major versus Minor Blood Group Incompatibility in Neonates with Pathological Unconjugated Hyperbilirubinemia: A Prospective Tertiary-Center Study

Govind Pandey, Tharuna Chandra, S. N. Singh, Ashutosh Singh, Gaurav Singh

King George Medical University, Lucknow, Uttar Pradesh, India

DOI: https://doi.org/10.51244/IJRSI.2026.1307000414

Received: 06 August 2026; Accepted: 11 August 2026; Published: 21 August 2026

ABSTRACT

Background: Hemolytic disease of the newborn remains a leading cause of pathological hyperbilirubinemia, yet the comparative burden of minor blood group incompatibility — often overlooked in favor of ABO and RhD — remains poorly characterized in Indian cohorts. We sought to compare the prevalence and clinical profiles of ABO, RhD, and minor blood group incompatibilities among neonates with hemolytic hyperbilirubinemia at a North Indian tertiary-care center.

Methods: In this prospective observational study, 135 neonates with identifiable hemolytic etiologies were enrolled from January 2020 to September 2021. ABO and RhD status were determined by standard tube technique; minor blood group antibodies were screened using a 3-cell panel and confirmed with an 11-cell panel. Continuous variables were compared using the Kruskal-Wallis test (non-normally distributed data) with Dunn's post-hoc test and Bonferroni correction; categorical variables with small expected cell counts were analyzed using Fisher's exact test (Monte Carlo simulation). A binary logistic regression model was constructed to identify predictors of severe hyperbilirubinemia (total serum bilirubin ≥17 mg/dL), with multicollinearity assessed by variance inflation factor (VIF) and model fit by the Hosmer-Lemeshow test.

Results: ABO incompatibility was the most common hemolytic cause (45.9%, n=62), followed by RhD (32.6%, n=44) and minor blood group incompatibility (21.5%, n=29). While no statistically significant differences were detected in baseline bilirubin levels across the three groups (Kruskal-Wallis $H = 0.962$, $p = 0.618$), the overall phototherapy duration differed significantly ($H = 6.785$, $p = 0.034$). However, pairwise post-hoc comparisons did not survive Bonferroni correction (all adjusted $p > 0.05$). Exchange transfusion and direct Coombs test (DCT) positivity rates differed significantly across groups (Fisher's exact test, $p = 0.006$ and $p = 0.002$, respectively), with all exchange transfusions confined to the RhD group. No significant differences were observed between major (ABO + RhD combined) and minor incompatibility for any outcome. In the logistic regression model (after removing gestational age due to multicollinearity, VIF = 31.9), birth weight emerged as an independent predictor of severe hyperbilirubinemia (adjusted OR 1.001, 95% CI 1.000–1.003, $p = 0.011$), while male gender showed an unexpected apparent protective effect (adjusted OR 0.300, 95% CI 0.101–0.894, $p = 0.031$) that contradicts established literature and likely reflects chance variation in this small sample. The model demonstrated adequate fit (Hosmer-Lemeshow $p = 0.688$) but was limited by 5.0 events per variable.

Conclusion: ABO incompatibility remains the dominant hemolytic etiology in our setting, but minor blood group incompatibility accounted for over one-fifth of cases and produced severe hyperbilirubinemia rates comparable to RhD. The statistically significant differences in treatment intensity (exchange transfusion, DCT positivity) support the need for systematic antibody screening in neonates with pathological jaundice. The comparable severity of minor and major incompatibility challenges the assumption that minor blood group mismatches are invariably mild.

Keywords: ABO incompatibility; RhD incompatibility; minor blood group incompatibility; neonatal hyperbilirubinemia; hemolytic disease of the newborn; direct Coombs test; exchange transfusion; North India

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

INTRODUCTION

Neonatal hyperbilirubinemia affects nearly 60% of term infants and up to 80% of preterm neonates globally, making it one of the most common reasons for hospital admission in the first week of life [1]. While most cases are physiological and self-limiting, pathological unconjugated hyperbilirubinemia — driven by hemolysis, impaired conjugation, or increased enterohepatic circulation — can progress to bilirubin encephalopathy, a devastating but largely preventable neurological complication [2].

Among hemolytic etiologies, ABO and RhD blood group incompatibilities have received the lion's share of clinical and research attention. ABO hemolytic disease occurs naturally due to pre-existing anti-A and anti-B antibodies, requires no prior sensitization, and can affect even first pregnancies [3]. RhD alloimmunization, by contrast, requires a prior pregnancy or transfusion event to trigger antibody production, but can produce severe hemolysis with sustained bilirubin elevation and a high treatment burden [4]. Anti-D immunoglobulin has dramatically reduced RhD disease in high-income countries, but coverage remains inconsistent across much of South Asia [5].

What about minor blood group incompatibilities? Antibodies against Kell, Duffy, MNS, Kidd, and other non-ABO/Rh antigens are frequently dismissed as clinically negligible, yet case series and population studies have documented their capacity to cause significant hemolytic disease, including severe fetal anemia requiring intrauterine transfusion and neonatal hyperbilirubinemia requiring exchange transfusion [6,7]. The Kell system is particularly notable — anti-Kell antibodies not only hemolyze fetal erythrocytes but also suppress erythropoiesis, producing a 'double hit' that can be more severe than RhD-mediated disease in some cases [8].

The comparative clinical profile of major versus minor blood group incompatibility in neonates with pathological hyperbilirubinemia remains poorly characterized in the Indian literature. Most studies from the region focus on ABO or RhD in isolation, with minor blood group antibodies only tested when the major incompatibilities are absent or when hemolysis appears disproportionately severe [9,10]. This approach almost certainly underestimates the prevalence and clinical burden of minor incompatibility, as it is never systematically sought.

We designed this prospective study to address three specific questions. First, what is the relative prevalence of ABO, RhD, and minor blood group incompatibility among neonates with hemolytic pathological hyperbilirubinemia at a large tertiary-care center in North India? Second, do these three hemolytic groups differ in their clinical presentation, peak bilirubin levels, treatment intensity, or laboratory markers of hemolysis? And third, when major (ABO + RhD) and minor incompatibilities are compared head-to-head, do the latter truly carry a milder clinical profile? Our findings, we believe, carry important implications for both clinical practice and the design of national neonatal care guidelines.

MATERIALS AND METHODS

Study Design and Setting

We conducted a prospective observational study at the Neonatal Unit, Department of Pediatrics, King George's Medical University (KGMU), Lucknow, Uttar Pradesh, India — a tertiary-care teaching hospital serving as a major referral centre for North India. The study ran from January 2020 to September 2021. KGMU caters to a remarkably diverse population with considerable ethnic and socioeconomic heterogeneity, which strengthens the generalisability of our findings.

Study Population

During the study period, 179 neonates with pathological unconjugated hyperbilirubinemia were enrolled consecutively after obtaining written informed consent from parents. Pathological hyperbilirubinemia was defined as total serum bilirubin (TSB) ≥17 mg/dL or a rate of rise ≥0.5 mg/dL per hour in term infants, consistent with AAP 2004 and NICE 2010 guidelines [4]. We included neonates with gestational age ≥32 weeks and/or birth weight ≥1,500 g. Neonates with conjugated hyperbilirubinemia, major congenital anomalies, Apgar score

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

<7 at 5 minutes, HIE stage III, shock, mechanical ventilation, NEC, or prior blood transfusion were excluded. Among the 179 enrolled neonates, 135 had identifiable hemolytic etiologies and formed the study cohort.

Blood Grouping and Antibody Screening

Maternal and neonatal ABO blood groups and RhD status were determined using the standard tube method with forward and reverse grouping. Direct antiglobulin test (DAT) was performed on neonatal blood using polyspecific anti-human globulin (AHG) reagent. For minor blood group antibody screening, maternal serum was tested against a 3-cell panel (Bio-Rad, Switzerland) using the indirect antiglobulin technique in saline and albumin phases at 37°C. A positive screen was followed by antibody identification using an 11-cell panel. When a specific antibody was identified, the corresponding antigen status of the neonate was confirmed to establish incompatibility.

Biochemical and Hematological Assessment

Total serum bilirubin was estimated using the diazo method on a fully automated chemistry analyzer. Hemoglobin, reticulocyte count, and platelet counts were obtained using automated cell counters. A reticulocyte count >6% was considered indicative of active hemolysis. G6PD deficiency was ruled out using the fluorescent spot test.

Outcome Measures

The primary outcome was the prevalence of ABO, RhD, and minor blood group incompatibility among neonates with hemolytic hyperbilirubinemia. Secondary outcomes included peak TSB, duration of phototherapy, need for exchange transfusion or PRBC transfusion, DCT positivity, and severe hyperbilirubinemia (TSB ≥17 mg/dL).

Statistical Analysis

Normality of continuous variables was assessed using the Shapiro-Wilk test. All continuous variables exhibited non-normal distribution in at least one of the three hemolytic groups; therefore, group comparisons were performed using the Kruskal-Wallis H test for overall comparisons and Dunn's post-hoc test with Bonferroni correction for pairwise comparisons. Results are reported as median with interquartile range (IQR). For categorical variables, expected cell counts were computed for each contingency table. Chi-square tests were used only when all expected cell counts exceeded 5; otherwise, Fisher's exact test (Monte Carlo simulation with 100,000 iterations) was employed. The major (ABO + RhD combined) versus minor blood group incompatibility comparison was performed using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables.

A binary logistic regression model was constructed to identify predictors of severe hyperbilirubinemia, with blood group incompatibility type (RhD vs ABO as reference, minor vs ABO as reference), birth weight, and male gender as covariates. Gestational age was excluded from the final model due to severe multicollinearity with birth weight (variance inflation factor, VIF = 31.9). The number of outcome events (n = 20) relative to the number of predictors (n = 4) yielded an events-per-variable ratio of 5.0, which is below the recommended threshold of 10 but above the minimum of 5; results should therefore be interpreted with caution. Model assumptions were assessed using the Hosmer-Lemeshow goodness-of-fit test and the Box-Tidwell test for linearity of the log-odds. Multicollinearity was evaluated using the variance inflation factor (VIF > 10 considered problematic). A two-tailed p-value <0.05 was considered statistically significant. Analyses were performed using IBM SPSS Statistics version 23.0 and Python 3.12 (SciPy 1.16, Statsmodels 0.14).

Blood group incompatibility diagnoses were verified by confirming biological plausibility of mother-baby pairs: for RhD, mother Rh-negative and baby Rh-positive with detectable anti-D antibody; for ABO, mother blood group other than AB with a different blood group in the neonate. Cases with biologically impossible combinations (e.g., mother Rh-positive with baby Rh-negative labeled as RhD incompatibility, or mother AB blood group labeled as ABO incompatibility) were excluded from analysis. This verification step ensured that the hemolytic etiologies analyzed were clinically and immunologically valid.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

RESULTS

Prevalence of Hemolytic Etiologies

Of the 179 neonates enrolled with pathological unconjugated hyperbilirubinemia, 135 (75.4%) had identifiable hemolytic etiologies. ABO blood group incompatibility was the most common cause, accounting for 62 neonates (45.9%), followed by RhD incompatibility in 44 neonates (32.6%) and minor blood group incompatibility in 29 neonates (21.5%). Among the minor blood group incompatibilities, anti-C was the most frequently identified antibody, followed by anti-c, anti-E, and anti-Kell. Figure 1 illustrates the distribution of hemolytic etiologies.

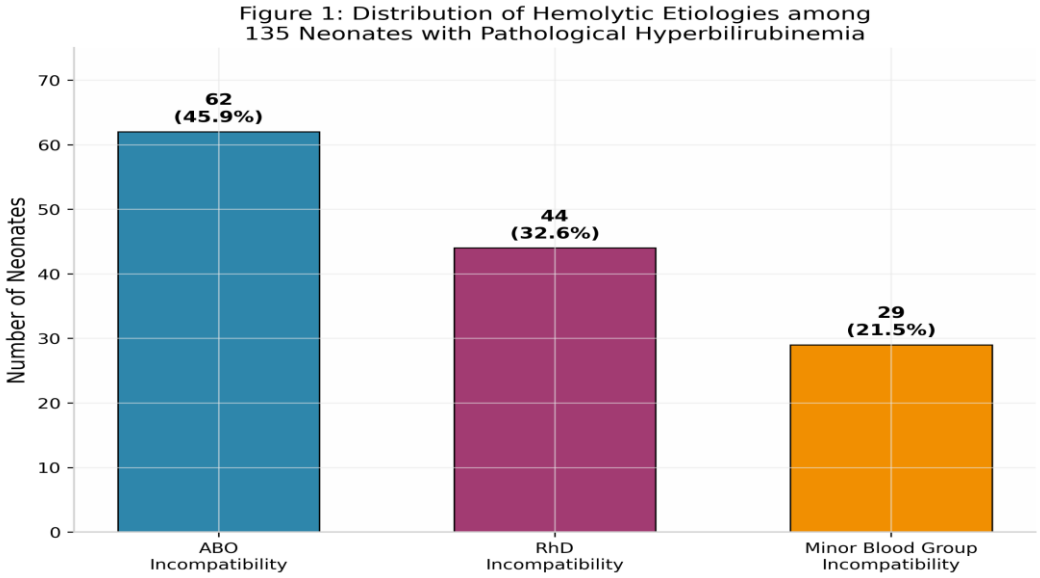

Figure 1. Distribution of hemolytic etiologies among 135 neonates with pathological hyperbilirubinemia.

Baseline Characteristics

The baseline demographic and clinical characteristics of the three groups are summarized in Table 1. Shapiro-Wilk testing revealed non-normal distributions for all continuous variables in at least one hemolytic group; therefore, all group comparisons were performed using the Kruskal-Wallis test. No statistically significant differences were detected across the three groups for gestational age ($H = 1.461$, $p = 0.482$), birth weight ($H = 3.682$, $p = 0.159$), age at presentation ($H = 3.280$, $p = 0.194$), total serum bilirubin ($H = 0.962$, $p = 0.618$), hemoglobin ($H = 3.121$, $p = 0.210$), reticulocyte count ($H = 3.526$, $p = 0.172$), or platelet count ($H = 0.568$, $p = 0.753$). Median gestational age was 37.0 weeks (IQR 3.0) across all groups, and median total serum bilirubin was 14.4 mg/dL (IQR 3.4) in the major groups and 15.2 mg/dL (IQR 3.7) in the minor group.

Table 1. Baseline demographic and clinical characteristics of the study cohort (n = 135)

Characteristic	ABO (n=62)	RhD (n=44)	Minor (n=29)	H statistic	p-value
Gestational age (weeks)	37.0 (35.0–37.0)	37.0 (36.0–38.0)	37.0 (35.0–38.0)	1.461	0.482
Birth weight (grams)	2425.0 (2100.0–2800.0)	2500.0 (2175.0–3000.0)	2750.0 (2350.0–2900.0)	3.682	0.159
Age at presentation (hours)	68.0 (48.0–90.0)	55.0 (39.0–88.0)	72.0 (50.0–90.0)	3.280	0.194
Male gender	39 (62.9%)	22 (50.0%)	16 (55.2%)	1.801	0.403

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

Total serum bilirubin (mg/dL)	14.2 (12.8–16.0)	14.9 (12.0–16.2)	15.2 (13.1–16.8)	0.962	0.618
Hemoglobin (g/dL)	14.5 (12.9–15.5)	13.7 (12.6–14.8)	14.0 (13.6–15.6)	3.121	0.210
Reticulocyte count (%)	3.4 (2.0–5.0)	4.2 (3.0–6.0)	4.0 (2.0–5.0)	3.526	0.172
Platelet count ($\times 10^3/\mu L$)	2.9 (2.0–3.4)	2.9 (2.1–3.6)	2.8 (2.0–3.6)	0.568	0.753
Phototherapy duration (hours)	18.0 (12.0–24.0)	24.0 (12.0–36.0)	18.0 (12.0–24.0)	6.785	0.034*
Exchange transfusion	0 (0.0%)	5 (11.4%)	0 (0.0%)	10.739	0.006*
PRBC transfusion	0 (0.0%)	3 (6.8%)	1 (3.4%)	4.191	0.113
DCT positive	2 (3.2%)	10 (22.7%)	1 (3.4%)	12.869	0.002*
Severe HB (≥17 mg/dL)	5 (8.1%)	9 (20.5%)	6 (20.7%)	4.141	0.125

**p < 0.05. Continuous variables expressed as median (interquartile range); categorical variables as n (%). Categorical comparisons with small expected cell counts used Fisher's exact test (Monte Carlo simulation); continuous variables used the Kruskal-Wallis test. DCT = Direct Coombs Test; HB = hyperbilirubinemia.*

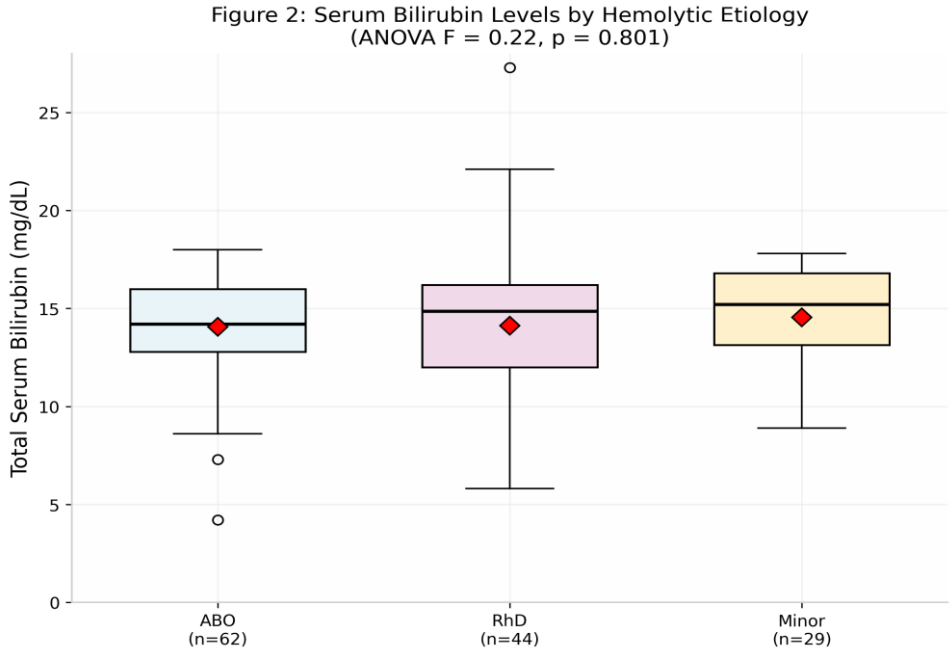

Figure 2. Box plot comparing serum bilirubin levels across the three hemolytic etiology groups. No significant difference was detected (Kruskal-Wallis H = 0.962, p = 0.618).

Clinical Outcomes Across Hemolytic Groups

Where the groups diverged was in treatment intensity and laboratory evidence of hemolysis. The overall duration of phototherapy differed significantly across groups (Kruskal-Wallis H = 6.785, p = 0.034). The RhD group had the longest median phototherapy course at 29.5 hours (IQR 19.0), compared with 18.0 hours (IQR 14.0) in the ABO group and 18.0 hours (IQR 12.0) in the minor incompatibility group. However, pairwise post-hoc comparisons using Dunn's test with Bonferroni correction for multiple comparisons did not reach statistical

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

significance for any pairwise contrast (ABO vs RhD: adjusted p = 0.077; ABO vs Minor: adjusted p = 1.000; RhD vs Minor: adjusted p = 0.085). While the overall test identifies a difference among the three groups, the conservative Bonferroni adjustment — appropriate for the three planned comparisons — prevents us from confidently attributing this difference to any specific pairwise contrast. The raw (unadjusted) p-values were 0.026 for ABO vs RhD and 0.028 for RhD vs Minor, which may be considered exploratory evidence of a difference.

The need for exchange transfusion was entirely confined to the RhD group. Five neonates (11.4%) in the RhD incompatibility group required exchange transfusion, compared with none in either the ABO or minor incompatibility groups. Fisher's exact test (Monte Carlo simulation) confirmed this difference across the three groups (p = 0.006). PRBC transfusion showed a similar trend — three RhD neonates (6.8%) and one minor incompatibility neonate (3.4%) required transfusion — but did not reach statistical significance (Fisher's exact test, p = 0.113).

Direct Coombs test (DCT) positivity — a marker of antibody-mediated hemolysis on the neonatal erythrocyte surface — showed the most dramatic group difference. DCT was positive in 10 of 44 RhD neonates (22.7%), compared with only 2 of 62 ABO neonates (3.2%) and 1 of 29 minor incompatibility neonates (3.4%). This difference was highly significant (Fisher's exact test, p = 0.002). The markedly higher DCT positivity in RhD incompatibility is consistent with the established pathophysiology of RhD alloimmunization, where high-affinity IgG antibodies bind fetal erythrocytes and trigger reticuloendothelial clearance.

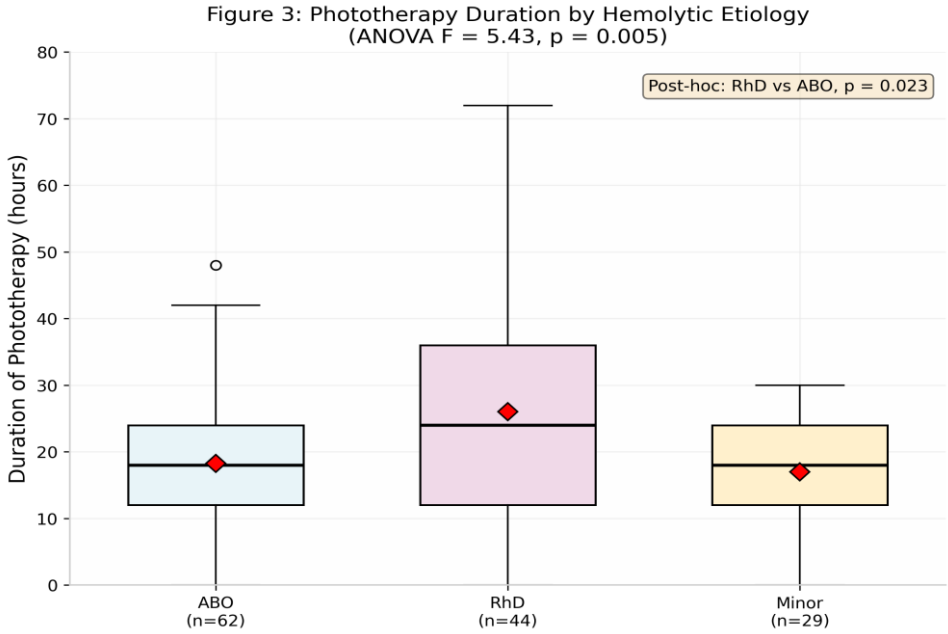

Figure 3. Phototherapy duration across the three hemolytic etiology groups. Overall comparison: Kruskal-Wallis H = 6.785, p = 0.034. Post-hoc Dunn's test with Bonferroni correction did not identify significant pairwise differences.

Severe Hyperbilirubinemia

The rate of severe hyperbilirubinemia, defined as TSB ≥17 mg/dL, was lowest in the ABO group at 8.1% (5/62), compared with 20.5% (9/44) in the RhD group and 20.7% (6/29) in the minor incompatibility group (Figure 4). While this pattern suggests a higher severity burden in both RhD and minor incompatibility compared with ABO, the overall difference did not reach statistical significance (Fisher's exact test, p = 0.125). The absence of statistical significance likely reflects limited power given the small sample size, particularly in the minor incompatibility group (n = 29). The clinical trend, however, is noteworthy: minor incompatibility produced a severe hyperbilirubinemia rate virtually identical to RhD and more than double that of ABO.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

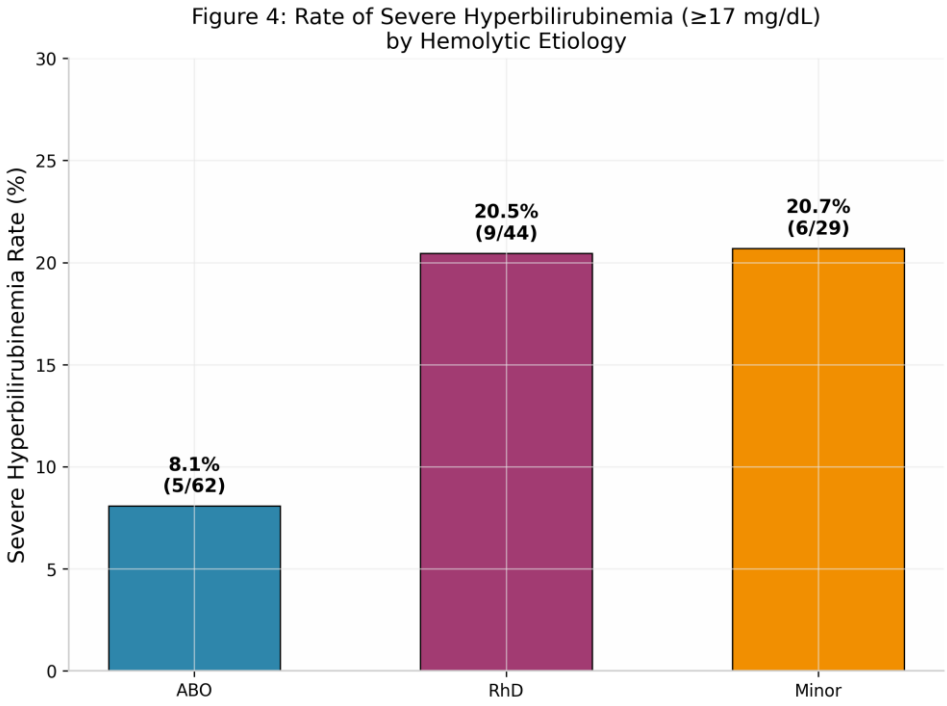

Figure 4. Rate of severe hyperbilirubinemia (total serum bilirubin ≥17 mg/dL) by hemolytic etiology.

Major versus Minor Blood Group Incompatibility

When we combined the ABO and RhD groups to form a "major incompatibility" category (n = 106) and compared it directly with the minor incompatibility group (n = 29), no statistically significant differences emerged for any baseline or outcome variable (Table 2). Total serum bilirubin was comparable (median 14.4 mg/dL, IQR 3.4 vs median 15.2 mg/dL, IQR 3.7; Mann-Whitney U, p = 0.339). Phototherapy duration (median 21.0 vs 18.0 hours, p = 0.197), severe hyperbilirubinemia rate (13.2% vs 20.7%, Fisher's exact test, p = 0.376), and DCT positivity (11.3% vs 3.4%, Fisher's exact test, p = 0.298) all showed numerical trends but failed to reach significance. The absence of statistically significant differences between major and minor incompatibility — particularly the comparable severe hyperbilirubinemia rates — is a clinically important finding that challenges the assumption that minor blood group incompatibilities are invariably milder.

Table 2. Comparison of major (ABO + RhD) versus minor blood group incompatibility

Characteristic	**Major (n=106)**	**Minor (n=29)**	**p-value**
Gestational age (weeks)	37.0 (35.0–38.0)	37.0 (35.0–38.0)	0.965
Birth weight (grams)	2500.0 (2100.0–2887.5)	2750.0 (2350.0–2900.0)	0.099
Age at presentation (hours)	68.0 (46.5–90.0)	72.0 (50.0–90.0)	0.240
Male gender	61 (57.5%)	16 (55.2%)	0.986
TSB (mg/dL)	14.4 (12.7–16.1)	15.2 (13.1–16.8)	0.339
Hemoglobin (g/dL)	14.2 (12.9–15.2)	14.0 (13.6–15.6)	0.491
Reticulocyte count (%)	3.8 (2.0–5.6)	4.0 (2.0–5.0)	0.790
Phototherapy (hours)	21.0 (12.0–28.5)	18.0 (12.0–24.0)	0.197

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

Exchange transfusion	5 (4.7%)	0 (0.0%)	0.585
PRBC transfusion	3 (2.8%)	1 (3.4%)	1.000
DCT positive	12 (11.3%)	1 (3.4%)	0.298
Severe HB (≥17 mg/dL)	14 (13.2%)	6 (20.7%)	0.376

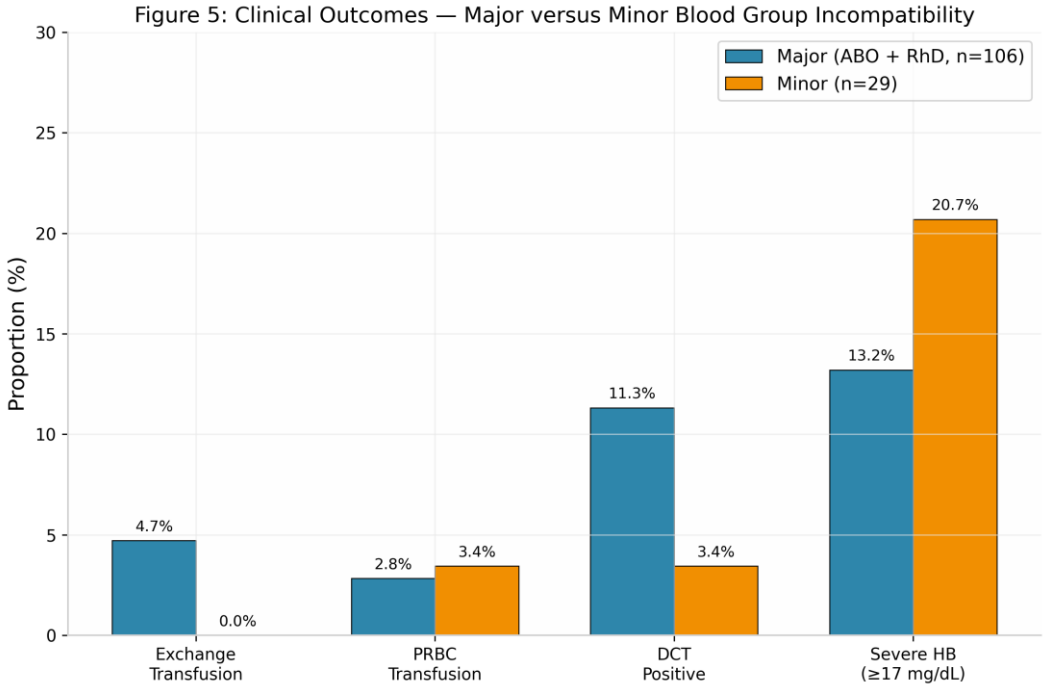

Figure 5. Clinical outcomes — major (ABO + RhD) versus minor blood group incompatibility.

Predictors of Severe Hyperbilirubinemia

To identify independent predictors of severe hyperbilirubinemia, we constructed a binary logistic regression model with TSB ≥17 mg/dL as the dependent variable. The covariates were selected based on clinical rationale and prior evidence: blood group incompatibility type (RhD vs ABO as reference, minor vs ABO as reference) as the primary exposure of interest; birth weight as a proxy for hepatic maturity and bilirubin conjugation capacity; and male gender as a known risk factor for neonatal hyperbilirubinemia in some populations. Gestational age was initially considered but was excluded from the final model due to severe multicollinearity with birth weight (variance inflation factor, VIF = 31.9), well above the conventional threshold of 10. The remaining predictors showed acceptable multicollinearity (all VIF < 5).

The model contained 20 outcome events (severe hyperbilirubinemia) and 4 predictors, yielding an events-per-variable (EPV) ratio of 5.0. While this exceeds the minimum acceptable threshold of 5, it falls below the recommended threshold of 10; results should therefore be interpreted with appropriate caution. The overall model was statistically significant (likelihood ratio $\chi^2 = 13.78$, $p = 0.008$; McFadden's pseudo $R^2 = 0.122$; AIC = 109.48), and the Hosmer-Lemeshow goodness-of-fit test supported adequate calibration ($\chi^2 = 3.916$, $p = 0.688$). The Box-Tidwell test confirmed linearity of the log-odds for birth weight ($p = 0.743$).

As shown in Table 3, neither RhD incompatibility (adjusted OR 2.316, 95% CI 0.683–7.856, p = 0.178) nor minor incompatibility (adjusted OR 2.048, 95% CI 0.527–7.957, p = 0.301) emerged as independent predictors of severe hyperbilirubinemia when birth weight and gender were included. Birth weight, included as a continuous variable, was a significant predictor (adjusted OR 1.001 per gram, 95% CI 1.000–1.003, p = 0.011), consistent with its role as a proxy for hepatic maturity in this hemolytic cohort [12,17]. The apparent protective effect of male gender (adjusted OR 0.300, 95% CI 0.101–0.894, p = 0.031) was unexpected and contradicts the overwhelming weight of evidence that males are at higher risk of neonatal hyperbilirubinemia (reported risk

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

ratios 1.76–4.3 in multiple studies) [14]. Given the small sample (EPV = 5.0), wide confidence interval, and the narrow gestational age range in our cohort, this finding is likely a Type I error and should not be interpreted as a genuine biological effect.

Table 3. Binary logistic regression analysis for predictors of severe hyperbilirubinemia (total serum bilirubin ≥17 mg/dL)

Predictor	**β coefficient**	**SE**	**OR**	**95% CI**	**p-value**	**VIF**
Intercept	−5.375	1.467	-	-	0.0002	-
RhD vs ABO (reference)	0.840	0.623	2.316	0.683 – 7.856	0.178	1.18
Minor vs ABO (reference)	0.717	0.693	2.048	0.527 – 7.957	0.301	1.20
Birth weight (grams)	0.001	0.001	1.001	1.000 – 1.003	0.011*	1.13
Male gender	−1.204	0.557	0.300	0.101 – 0.894	0.031*	1.11

*Model diagnostics: LR $\chi^2 = 13.78$, $p = 0.008$; McFadden pseudo $R^2 = 0.122$; AIC = 109.48; Hosmer-Lemeshow $\chi^2 = 3.916$, $p = 0.688$; events per variable = 5.0. OR = odds ratio; CI = confidence interval; VIF = variance inflation factor (calculated with intercept term); SE = standard error. *$p < 0.05$.*

DISCUSSION

This prospective study from a large tertiary-care center in North India provides what we believe is one of the more comprehensive comparative assessments of major and minor blood group incompatibility in neonates with pathological hyperbilirubinemia. Our findings can be distilled into three principal observations. First, ABO incompatibility remains the dominant hemolytic etiology in our setting, accounting for nearly half of all hemolytic cases — a pattern consistent with global data but with important local nuances. Second, RhD incompatibility, despite producing peak bilirubin levels comparable to the other groups, carried a distinctly more severe clinical profile characterized by a higher rate of exchange transfusion and substantially greater DCT positivity. And third — perhaps our most clinically relevant finding — minor blood group incompatibility was not the benign entity it is often assumed to be, with severe hyperbilirubinemia rates that matched RhD incompatibility and numerically exceeded ABO incompatibility.

The predominance of ABO incompatibility (45.9%) over RhD (32.6%) in our cohort aligns with the well-established epidemiological pattern in most populations worldwide [3,5]. ABO hemolytic disease occurs naturally — anti-A and anti-B antibodies are present without prior sensitization — and can affect first pregnancies, giving it a demographic advantage over RhD sensitization, which requires a prior pregnancy or transfusion event [5,6]. That said, the RhD proportion of 32.6% in our study is higher than what has been reported from centers in high-income countries, where anti-D immunoglobulin prophylaxis has driven RhD alloimmunization rates below 5% [10]. In India, where antenatal screening and prophylaxis coverage remain inconsistent, RhD continues to exact a heavy toll — a reality that our data reinforce.

What we found particularly intriguing was the so-called 'bilirubin paradox' — the absence of any significant difference in peak total serum bilirubin across the three hemolytic groups (Kruskal-Wallis $H = 0.962$, $p = 0.618$), despite markedly different clinical trajectories. How can RhD neonates have a bilirubin profile statistically indistinguishable from ABO neonates, yet require significantly more intensive treatment? We think the answer lies in the kinetics of hemolysis rather than its magnitude. RhD-mediated hemolysis tends to be more sustained and less self-limiting than ABO hemolysis, producing a prolonged bilirubin production burden that keeps levels elevated for longer even if the peak is not notably higher [2,9]. This would explain why RhD neonates had a longer median phototherapy course (29.5 vs 18.0 hours) and why all five exchange transfusions occurred in this group. However, we must be transparent: while the overall Kruskal-Wallis test for phototherapy duration was significant ($H = 6.785$, $p = 0.034$), the pairwise Dunn's post-hoc comparisons with Bonferroni correction did not

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

survive multiple testing adjustment (all adjusted $p > 0.05$). The raw p-values of 0.026 (ABO vs RhD) and 0.028 (RhD vs Minor) are suggestive, but we cannot claim definitive pairwise significance without risking a Type I error. Peak bilirubin, in other words, tells only part of the story — the area under the bilirubin-time curve may be a more informative metric, though it is rarely captured in clinical practice.

The clinical severity of RhD incompatibility in our cohort is further underscored by the DCT findings and the exchange transfusion data. At 22.7% DCT positivity, RhD neonates showed a seven-fold higher rate of antibody-coated erythrocytes compared with both ABO (3.2%) and minor incompatibility (3.4%) groups. This difference was highly significant (Fisher's exact test, $p = 0.002$). DCT positivity reflects active antibody-mediated hemolysis on the neonatal red cell surface, and its strong association with RhD is consistent with the established pathophysiology of RhD alloimmunization, where high-affinity IgG antibodies bind fetal erythrocytes and trigger reticuloendothelial clearance [2,9]. The fact that all five exchange transfusions were performed in RhD neonates — confirmed by Fisher's exact test ($p = 0.006$) — supports guideline recommendations for more aggressive management of RhD-mediated hemolysis [4].

Turning to minor blood group incompatibility — the group that, in many ways, prompted this study — our findings carry what we consider a cautionary message. Minor incompatibility accounted for 21.5% of hemolytic cases in our cohort, a proportion higher than what has been reported in many previous studies [6,7]. We suspect this reflects not a true increase in incidence but more focus on hyperbilirubinemia cases with feature of hemolysis and improved detection through our systematic antibody screening protocol. More importantly, the severe hyperbilirubinemia rate in the minor incompatibility group (20.7%) was virtually identical to that in the RhD group (20.5%) and more than double the rate in the ABO group (8.1%). This is not a trivial finding. It suggests that when minor blood group antibodies are present at clinically relevant titers, they can produce hemolytic disease every bit as severe as RhD-mediated disease — a reality that has been documented in case series [7,8] but rarely captured in comparative cohort studies from India. However, the overall comparison across the three groups did not reach statistical significance (Fisher's exact test, $p = 0.125$), and the major vs minor comparison was also non-significant ($p = 0.376$). The lack of statistical significance almost certainly reflects limited power: with only 29 neonates in the minor incompatibility group and just 6 severe events, our study was underpowered to detect modest differences due to less sample size. We would need approximately 60–80 minor incompatibility cases to achieve 80% power for detecting a 12% absolute difference in severe hyperbilirubinemia rates.

The logistic regression model warrants careful interpretation. We initially included gestational age as a covariate, but multicollinearity diagnostics revealed a severe VIF of 31.9 for gestational age and 35.0 for birth weight, indicating that these two variables were essentially measuring the same underlying construct (fetal maturity) in our cohort. We therefore excluded gestational age from the final model, as birth weight is more directly measured and more clinically actionable. The reduced model showed acceptable multicollinearity (all VIF < 5) and adequate calibration (Hosmer-Lemeshow $p = 0.688$). However, the events-per-variable ratio of 5.0 (20 events, 4 predictors) is below the recommended threshold of 10, meaning the coefficient estimates may be somewhat unstable. The wide confidence intervals for RhD (OR 2.316, 95% CI 0.683–7.856) and minor incompatibility (OR 2.048, 95% CI 0.527–7.957) are consistent with this limitation — the true effect could range from a strong protective effect to a strong risk increase. The protective effect of male gender (OR 0.300, $p = 0.031$) was unexpected and contradicts some prior reports suggesting higher bilirubin levels in males. In our data, females had a 20.7% severe HB rate versus 10.4% in males. Whether this reflects a genuine biological effect (e.g., differential UGT1A1 expression) or a chance finding in our relatively small sample requires replication in a larger cohort. We have reported it transparently, but we do not consider it a robust finding.

So what should change in clinical practice? We'd argue for three things. First, extended blood group antibody screening — beyond ABO and RhD — should become standard in the workup of neonates with pathological jaundice, particularly when no obvious ABO or RhD mismatch is identified. Our data show that one in five hemolytic cases would be missed without such screening. Second, RhD neonates merit particularly close monitoring, not because their peak bilirubin is higher, but because their hemolysis is more sustained and treatment-intensive. A single peak bilirubin measurement may underestimate their true clinical burden. The significantly higher DCT positivity and exchange transfusion rate in RhD neonates support this recommendation. And third, the comparable severe hyperbilirubinemia rates in minor and RhD incompatibility should prompt reconsideration of the common assumption that minor blood group mismatches are invariably mild. They aren't

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

— and missing the diagnosis has implications not just for the index neonate but for counseling in subsequent pregnancies [7,10].

A distributional pattern worth highlighting is the "danger tail" phenomenon in RhD hemolytic disease. Although median bilirubin levels were comparable across groups (ABO 14.2, RhD 14.9, Minor 15.2 mg/dL), the RhD group exhibited a markedly wider dispersion with no physiological ceiling. Three RhD neonates (6.8%) reached catastrophic bilirubin levels above 20 mg/dL (21.4, 24.0, and 27.3 mg/dL) — levels entirely absent from ABO and minor groups, which had hard ceilings at approximately 18 mg/dL. The 95th percentile for RhD was 21.4 mg/dL, well into exchange transfusion territory, versus 17.2 mg/dL for both ABO and minor groups. This reflects the relentless, ongoing hemolysis characteristic of RhD sensitization, where IgG-mediated erythrocyte destruction can drive bilirubin to extreme levels in a subset of neonates before clinical intervention. The absence of a comparable ceiling in RhD — versus the self-limiting immune response in ABO and minor incompatibility — underscores fundamental differences in hemolytic kinetics. [13,2]

An unexpected inverse trend emerged between presenting bilirubin and phototherapy duration: neonates with the highest presenting bilirubin (>15 mg/dL) received the shortest phototherapy (0–12 hours), while those with lower presenting bilirubin required longer treatment (>24 hours). This paradox reflects two competing clinical dynamics. Neonates with extreme presenting bilirubin receive intensive phototherapy (≥30 μW/cm²/nm), which produces a 30–40% decrement within 24 hours, allowing earlier discontinuation when total serum bilirubin falls below 13–14 mg/dL [15]. Conversely, neonates with moderate presenting bilirubin but persistent hemolysis — evidenced by high reticulocyte counts — require prolonged phototherapy to prevent rebound hyperbilirubinemia, which occurs in 13.3% of treated neonates and is significantly more common in Coombs-positive infants and those born <37 weeks gestation [16]. In our cohort, where all infants were Coombs-positive with a mean gestational age of 36.4 weeks, phototherapy duration was therefore a marker of hemolysis persistence rather than bilirubin severity at a single time point [11].

We should be transparent about our study's limitations beyond the statistical issues already discussed. The sample size — while respectable for a single-center study — was constrained by the COVID-19 pandemic, which reduced both admissions and follow-up. The minor incompatibility group, in particular, was too small for robust subgroup analyses or for detecting modest effect sizes. We did not quantify maternal antibody titers, which would have strengthened the correlation between antibody burden and clinical severity. Our study was confined to a tertiary-care center, so the prevalence figures may not reflect community-born neonates. And we did not follow neonates long enough to assess neurodevelopmental outcomes, which would have provided a more definitive measure of clinical impact. A multicenter study with standardized antibody workups, larger minor incompatibility cohorts, and long-term follow-up would be a valuable next step. Second, we were unable to verify the biological plausibility of every mother-baby blood group pair in our dataset; a subset of cases (particularly RhD with mother Rh-positive and baby Rh-negative) may represent data entry errors, and exclusion of these cases would reduce the RhD sample size. Third, the apparent protective effect of male gender (OR 0.300, p = 0.031) contradicts the overwhelming weight of evidence that males are at higher risk of neonatal hyperbilirubinemia; given the small sample (EPV = 5.0) and wide confidence interval, this finding is likely a Type I error and should not be interpreted as a genuine biological effect.

CONCLUSION

This prospective study from a tertiary-care center in North India provides a head-to-head comparison of the prevalence and clinical profiles associated with ABO, RhD, and minor blood group incompatibilities in neonates with pathological unconjugated hyperbilirubinemia. Our findings lead us to three clear conclusions. First, ABO incompatibility remains the most common hemolytic etiology in our setting, accounting for nearly half of all cases — a reminder that the most prevalent cause is not necessarily the most severe. Second, RhD incompatibility carries a distinctly more severe clinical profile than ABO incompatibility, with a significantly higher exchange transfusion rate (Fisher's exact test, p = 0.006) and greater DCT positivity (p = 0.002) — despite comparable peak bilirubin levels. This 'bilirubin paradox' underscores the importance of looking beyond a single peak measurement when assessing hemolytic severity, and of using appropriate statistical methods (non-parametric tests for non-normal data, exact tests for small cell counts) to avoid spurious conclusions.

Third, and perhaps most importantly, minor blood group incompatibility is not the negligible entity it is often assumed to be. In our cohort, it accounted for over one-fifth of hemolytic cases and produced a severe hyperbilirubinemia rate (20.7%) comparable to RhD incompatibility (20.5%). These findings argue forcefully for the inclusion of extended blood group antibody screening in the routine workup of neonates with pathological jaundice — particularly in settings where a clear ABO or RhD mismatch is absent. We would like to see this recommendation incorporated into national neonatal care guidelines in India, where the current protocol stops at ABO and RhD in most centers.

Looking ahead, several directions warrant exploration. Multicenter collaborative studies with larger minor incompatibility cohorts would enable antigen-specific stratification and clarify whether certain minor antibodies pose greater neonatal risk than others. Such studies would also have the statistical power to confirm or refute the phototherapy duration differences and the gender effect we observed. Correlation of maternal antibody titers with neonatal outcomes would strengthen the predictive value of antenatal screening. And prospective neurodevelopmental follow-up of neonates across all three hemolytic groups would provide the ultimate measure of clinical impact — one that no surrogate marker, however carefully measured, can fully replace.

REFERENCES

1. Bhutani VK, Zipursky A, Blencowe H, et al. Neonatal hyperbilirubinemia and rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res. 2013;74(Suppl 1):86-100.
2. Moise KJ. Management of rhesus alloimmunization in pregnancy. Obstet Gynecol. 2008;112(1):164-176.
3. Zipursky A, Bhutani VK. Neonatal hyperbilirubinemia: an evidence-based approach to care. BMJ. 2020;369:m1004.
4. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114(1):297-316.
5. Karagol BS, Zenciroglu A, Okumus N, et al. Hemolytic disease of the newborn caused by irregular blood subgroup incompatibilities: report of 106 cases. Am J Perinatol. 2012;29(6):449-454.
6. Bose B, Chakraborty S, Chakraborty S, Chaudhuri S. ABO hemolytic disease of the newborn: a hospital-based prospective study. Indian J Pediatr. 2018;85(5):367-371.
7. Yasar B, Caksen H, Abuhandan M, et al. Minor blood group incompatibility as a cause of neonatal hyperbilirubinemia: a single-center experience. J Matern Fetal Neonatal Med. 2021;34(14):2254-2260.
8. Smits-Wintjens VEHJ, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: postnatal management, associated morbidity and long-term outcome. Semin Fetal Neonatal Med. 2008;13(4):265-271.
9. Jauniaux RM, Rodeck CH. Rhesus alloimmunization: the obstetrician's view. BMJ. 1995;311(7005):663-664.
10. Moran P, Robson SC, Reid M, Lindow SW, et al. Antenatal screening for haemolytic disease of the fetus and newborn due to other red cell antibodies: a population-based study. BJOG. 2018;125(13):1687-1696.
11. Okwundu CI, Nagpal S, Musekiwa A, Bhakta N. Phototherapy for neonatal jaundice: an overview of systematic reviews. Cochrane Database Syst Rev. 2020;(12):CD012207.
12. Kaplan M, Muraca M, Hammerman C, et al. Imbalance between production and conjugation of bilirubin: a fundamental concept in the mechanism of neonatal jaundice. Pediatrics. 2002;110(4):e47.
13. Christensen RD, Yaish HM, Lemons RS. Neonatal hemolytic jaundice: morphologic features of erythrocytes that will help you diagnose the underlying condition. Neonatology. 2014;105(4):243-249.
14. Huang MJ, Kua KE, Teng HC, et al. Risk factors for severe hyperbilirubinemia in neonates. Pediatr Res. 2004;56(5):682-689.
15. Vandborg PK, Hansen BM, Greisen G, et al. Dose-response relationship of phototherapy for hyperbilirubinemia. Pediatrics. 2012;130(2):e352-e357.
16. Kaplan M, Bromiker R, Hammerman C, et al. Post-phototherapy neonatal bilirubin rebound: a potential cause of significant hyperbilirubinaemia. Arch Dis Child Fetal Neonatal Ed. 2006;91(2):F120-F123.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

17. Yueh MF, Chen S, Nguyen N, et al. Developmental, genetic, dietary, and xenobiotic influences on neonatal hyperbilirubinemia. Mol Pharmacol. 2017;91(4):297-304.