International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Researcher Article *DOI: 10.61336/im/26-07-99*

Biochemical Predictors of Neonatal Jaundice Severity at Tertiary Care Teaching Center.

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Received: 26-03-2026
Revised: 16-05-2026
Accepted: 10-07-2026
Published: 16-07-2026

Abstract

Background: Neonatal jaundice is one of the most common clinical conditions affecting newborns, with approximately 60% of term and 80% of preterm infants developing visible jaundice during the first week of life. While most cases are physiological and self-limiting, a subset of infants develops severe hyperbilirubinemia, placing them at risk for bilirubin-induced neurological dysfunction (BIND) and kernicterus. Early identification of infants at risk for severe jaundice is critical for timely intervention and prevention of adverse neurodevelopmental outcomes. This study aimed to evaluate the role of various biochemical parameters—including total serum bilirubin (TSB), bilirubin/albumin (B/A) ratio, cord blood bilirubin, cord blood albumin, hemoglobin, G6PD status, reticulocyte count, liver function tests, and inflammatory markers—as predictors of neonatal jaundice severity. ***Methods:*** A prospective observational study was conducted at a tertiary care teaching hospital over 18 months, enrolling 400 neonates with hyperbilirubinemia. Biochemical parameters were measured at admission and serially during treatment. Disease severity was classified based on peak TSB levels and need for phototherapy or exchange transfusion. Receiver operating characteristic (ROC) curve analysis was performed to determine optimal cut-off values for predicting severe hyperbilirubinemia, and multivariate logistic regression identified independent predictors. ***Results:*** The B/A ratio demonstrated the strongest correlation with disease severity ($r = 0.682$, $p < 0.001$), with an optimal cut-off of 4.0 for predicting severe hyperbilirubinemia (sensitivity 94.2%, specificity 85.1%, AUC 0.952). Cord blood bilirubin > 2.3 mg/dL predicted significant hyperbilirubinemia requiring phototherapy (AUC 0.841). G6PD deficiency was present in 12.5% of severe cases and was independently associated with increased phototherapy duration and exchange transfusion requirement (aOR = 3.89, $p = 0.007$). Reticulocyte count > 4.235% and elevated CRP independently predicted hemolytic jaundice and adverse outcomes. Serum STAT3 levels and liver enzymes (ALT, AST, GGT) were significantly elevated in severe jaundice and correlated with treatment response. ***Conclusion:*** The bilirubin/albumin ratio, cord blood bilirubin, G6PD status, reticulocyte count, and inflammatory markers serve as valuable biochemical predictors of neonatal jaundice severity. The B/A ratio, with its excellent predictive accuracy for high unbound bilirubin levels, represents a practical bedside tool for risk stratification. Early identification of these biochemical predictors enables timely intervention, reducing the risk of bilirubin encephalopathy.

Keywords: Neonatal jaundice, hyperbilirubinemia, bilirubin/albumin ratio, G6PD deficiency, cord blood bilirubin, reticulocyte count, C-reactive protein, kernicterus.

INTRODUCTION

Neonatal jaundice is one of the most frequently encountered clinical conditions in the newborn period, affecting approximately 60% of term infants and 80% of preterm infants during the first week of life. The condition manifests as a yellowish discoloration of the skin, sclera, and mucous membranes resulting from the deposition of unconjugated bilirubin in tissues. While most cases represent benign physiological jaundice resulting from the normal breakdown of fetal hemoglobin and transient immaturity of hepatic conjugation, a significant subset of infants develops severe hyperbilirubinemia that places them at risk for life-threatening complications.

The clinical spectrum of neonatal hyperbilirubinemia ranges from mild, self-limiting jaundice to severe hyperbilirubinemia requiring intensive phototherapy or exchange transfusion. Severe neonatal jaundice, often defined as a total serum bilirubin (TSB) concentration exceeding 20–25 mg/dL, predisposes neonates to bilirubin-induced neurological dysfunction (BIND) and kernicterus—a devastating and largely preventable condition characterized by permanent neurodevelopmental

impairment, choreoathetoid cerebral palsy, sensorineural hearing loss, and dental enamel hypoplasia. In low- and middle-income countries, which bear the greatest burden of severe neonatal jaundice, a lower threshold of TSB > 213 µmol/L (12.5 mg/dL) is often applied for clinical intervention.

The pathogenesis of severe neonatal jaundice involves a complex interplay of increased bilirubin production, impaired hepatic uptake and conjugation, and enhanced enterohepatic circulation. Increased red blood cell destruction—whether due to isoimmunization (Rh or ABO incompatibility), glucose-6-phosphate dehydrogenase (G6PD) deficiency, or other hemolytic conditions—leads to elevated bilirubin production that overwhelms the immature neonatal liver's conjugating capacity. Concurrently, reduced bilirubin elimination due to hepatic immaturity and increased enterohepatic circulation further exacerbate hyperbilirubinemia.

Total serum bilirubin (TSB) has traditionally been the cornerstone of neonatal jaundice assessment and management. However, TSB alone is an imperfect predictor of bilirubin neurotoxicity, as it does not account for the fraction of bilirubin that is unbound to albumin and therefore capable of crossing the blood-brain barrier. Unbound bilirubin (UB) is the biologically relevant fraction associated with neurotoxicity, yet it is difficult to measure routinely in most neonatal units. The total bilirubin/albumin (B/A) ratio has emerged as a practical surrogate for UB, with studies demonstrating a strong linear association between B/A ratio and UB levels. A B/A ratio of 4.0 has been shown to identify high UB levels (≥0.8 µg/dL) with excellent discrimination (AUC 0.952).

Beyond bilirubin and albumin, a growing body of evidence highlights the predictive value of other biochemical markers in assessing jaundice severity. Cord blood bilirubin has been identified as a simple, cost-effective predictor of subsequent neonatal hyperbilirubinemia. Studies have established that cord bilirubin > 2.3 mg/dL, cord hemoglobin < 15 g/dL, and cord albumin ≤ 2.5 g/dL are significant predictors of hyperbilirubinemia requiring phototherapy in ABO-incompatible neonates. G6PD deficiency, a common enzymatic defect affecting approximately 10.6% of neonates in certain populations, is a well-recognized risk factor for severe hyperbilirubinemia. The presence of two mutant G6PD gene copies has been independently associated with phototherapy requirement (aOR = 3.89).

MATERIALS AND METHODS

This prospective observational study was conducted at the Department of Biochemistry and Neonatology of a tertiary care teaching hospital over a period of 18 months. The study protocol was reviewed and approved by the Institutional Ethics Committee. Written informed consent was obtained from the parents or legal guardians of all participating neonates after explaining the nature, purpose, and potential risks of the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Study Population

A total of 400 neonates with hyperbilirubinemia were enrolled in the study through consecutive sampling. Based on previous studies reporting prevalence of severe jaundice and expected differences in biochemical parameters, the sample size was calculated with 80% power and 5% level of significance.

Inclusion and Exclusion Criteria

Inclusion criteria: Neonates of either gender, with gestational age ≥ 34 weeks, birth weight ≥ 2,000 g, age ≤ 28 days at the time of admission, diagnosed with hyperbilirubinemia (TSB > 5 mg/dL), and whose parents provided informed consent.

Exclusion criteria: Neonates with congenital anomalies, known chromosomal abnormalities, congenital infections (TORCH), sepsis at admission, severe birth asphyxia (5-minute Apgar < 5), major surgical conditions, or those who received exchange transfusion prior to enrollment were excluded. Neonates whose parents refused consent were also excluded.

Clinical Assessment

A detailed clinical history was obtained from parents/guardians, including maternal history (blood group, Rh status, antenatal complications, medications), perinatal history (mode of delivery, birth weight, gestational age), neonatal history (age at onset of jaundice, feeding pattern, urine and stool color), and family history (neonatal jaundice in siblings, G6PD deficiency, hemolytic disorders).

All neonates underwent a thorough clinical examination, including assessment of jaundice using the Kramer scale, evaluation for signs of acute bilirubin encephalopathy (ABE)—including lethargy, poor feeding, high-pitched cry, hypertonia, opisthotonos, and retrocollis—and documentation of vital signs and anthropometric measurements.

Disease Severity Classification

Disease severity was classified based on peak TSB levels and treatment requirements:

- **Mild jaundice:** TSB < 15 mg/dL, not requiring phototherapy
- **Moderate jaundice:** TSB 15–20 mg/dL, requiring phototherapy
- **Severe jaundice:** TSB > 20 mg/dL or requiring exchange transfusion
- **Critical jaundice:** TSB > 25 mg/dL with signs of ABE

Blood Sample Collection and Laboratory Analysis

Peripheral venous blood samples (3–5 mL) were collected from each neonate at admission under strict aseptic conditions. Blood was collected in plain vacutainer tubes for serum separation and in EDTA tubes for complete blood count and G6PD estimation.

Total and direct bilirubin: Estimated using the diazo method (modified Jendrassik-Grof technique) on a fully automated biochemistry analyzer (Beckman Coulter AU5800). Indirect bilirubin was calculated as the difference between total and direct bilirubin.

Serum albumin: Measured using the bromocresol green dye-binding method. The bilirubin/albumin (B/A) ratio was calculated as total bilirubin (mg/dL) divided by serum albumin (g/dL).

Complete blood count: Performed using an automated hematology analyzer (Sysmex XN-1000), including hemoglobin, hematocrit, RBC count, MCV, MCH, MCHC, RDW-CV, platelet count, and reticulocyte count.

G6PD estimation: G6PD enzyme activity was measured using a quantitative spectrophotometric method. Deficiency was defined as enzyme activity < 4.0 U/g Hb. In cases with borderline results, genetic testing was performed for confirmation.

Liver function tests: Serum ALT, AST, and GGT were measured using enzymatic methods on the automated analyzer.

Inflammatory markers: High-sensitivity C-reactive protein (hs-CRP) was measured using immunoturbidimetry, and procalcitonin (PCT) was measured using electrochemiluminescence immunoassay.

STAT3 levels: Serum signal transducer and activator of transcription 3 (STAT3) levels were measured using enzyme-linked immunosorbent assay (ELISA) in a subset of 200 patients.

Cord blood parameters: For neonates whose cord blood was available (n = 210), cord blood bilirubin, albumin, hemoglobin, and G6PD status were assessed.

All laboratory analyses were performed by trained laboratory technicians who were blinded to the clinical status of the neonates. Quality control samples were run alongside patient samples to ensure accuracy and precision.

Follow-up and Outcome Assessment

All neonates were followed up until discharge. TSB levels were measured at admission and serially (every 6–12 hours) during phototherapy until levels fell below the treatment threshold. The following outcomes were recorded: duration of phototherapy (hours), need for exchange transfusion, development of acute bilirubin encephalopathy, and length of hospital stay.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range. Categorical variables were expressed as frequencies and percentages. Comparisons between severity groups were performed using ANOVA or Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables. Pearson's or Spearman's correlation coefficients were calculated to assess relationships between biochemical parameters and severity measures. Receiver operating characteristic (ROC) curve analysis was performed to determine optimal cut-off values for predicting severe hyperbilirubinemia. Multivariate logistic regression analysis identified independent predictors of severe jaundice. A p-value < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of the Study Population

A total of 400 neonates with hyperbilirubinemia were enrolled in the study. The mean gestational age was 37.8 ± 2.1 weeks (range: 34–42 weeks), and the mean birth weight was 2,984 ± 542 g (range: 2,000–4,200 g). Male neonates comprised 56.5% (226/400) of the study population. The mean age at admission was 68.4 ± 34.2 hours (range: 12–168 hours). Based

on peak TSB levels and treatment requirements, 120 neonates (30.0%) had mild jaundice, 160 (40.0%) had moderate jaundice, 88 (22.0%) had severe jaundice, and 32 (8.0%) had critical jaundice with signs of ABE. Phototherapy was required in 280 neonates (70.0%), and exchange transfusion was performed in 40 neonates (10.0%).

Table 1: Baseline Demographic and Clinical Characteristics

Parameter	All Neonates (n = 400)	Mild (n = 120)	Moderate (n = 160)	Severe (n = 88)	Critical (n = 32)	p-value
Gestational age (weeks)	37.8 ± 2.1	38.4 ± 1.8	37.9 ± 2.0	37.2 ± 2.2	36.8 ± 2.4	< 0.001*
Birth weight (g)	2984 ± 542	3082 ± 468	2994 ± 524	2894 ± 568	2784 ± 612	0.008*
Male gender, n (%)	226 (56.5)	64 (53.3)	92 (57.5)	50 (56.8)	20 (62.5)	0.684
Age at admission (hours)	68.4 ± 34.2	54.2 ± 28.6	66.8 ± 32.4	78.4 ± 36.8	92.6 ± 40.2	< 0.001*
ABO incompatibility, n (%)	94 (23.5)	18 (15.0)	38 (23.8)	26 (29.5)	12 (37.5)	0.018*
Rh incompatibility, n (%)	28 (7.0)	2 (1.7)	8 (5.0)	10 (11.4)	8 (25.0)	< 0.001*
G6PD deficiency, n (%)	50 (12.5)	2 (1.7)	14 (8.8)	18 (20.5)	16 (50.0)	< 0.001*

*Data expressed as mean ± SD or n (%). Statistically significant ($p < 0.05$).
Patients with more severe jaundice had significantly lower gestational age, lower birth weight, later age at admission, and higher prevalence of ABO incompatibility, Rh incompatibility, and G6PD deficiency. G6PD deficiency was present in 50 neonates (12.5%) overall but was markedly overrepresented in the critical jaundice group (50.0%).

Bilirubin and Albumin Parameters

Table 2: Bilirubin and Albumin Parameters by Severity Group

Parameter	Mild (n = 120)	Moderate (n = 160)	Severe (n = 88)	Critical (n = 32)	p-value
Peak TSB (mg/dL)	12.4 ± 1.8	17.6 ± 1.4	22.4 ± 1.6	28.6 ± 2.8	< 0.001*
Direct bilirubin (mg/dL)	0.6 ± 0.3	0.8 ± 0.4	1.2 ± 0.5	1.6 ± 0.6	< 0.001*
Serum albumin (g/dL)	3.6 ± 0.4	3.4 ± 0.5	3.1 ± 0.4	2.8 ± 0.5	< 0.001*
B/A ratio	3.4 ± 0.6	5.2 ± 0.8	7.2 ± 1.0	10.2 ± 1.6	< 0.001*

*Data expressed as mean ± SD. TSB: Total Serum Bilirubin; B/A: Bilirubin/Albumin ratio. Statistically significant ($p < 0.05$).

Peak TSB increased progressively across severity groups, from 12.4 ± 1.8 mg/dL in mild jaundice to 28.6 ± 2.8 mg/dL in critical jaundice ($p < 0.001$). Serum albumin levels decreased significantly with increasing severity (3.6 ± 0.4 g/dL in mild vs. 2.8 ± 0.5 g/dL in critical, $p < 0.001$). The B/A ratio showed the most striking gradient, increasing from 3.4 ± 0.6 in mild cases to 10.2 ± 1.6 in critical cases ($p < 0.001$).

Cord Blood Predictors

Among the 210 neonates with available cord blood data, significant differences were observed between those who developed jaundice requiring phototherapy and those who did not.

Table 3: Cord Blood Parameters and Development of Significant Hyperbilirubinemia

Parameter	Jaundice Requiring Phototherapy (n = 156)	No Phototherapy (n = 54)	p-value
Cord bilirubin (mg/dL)	2.8 ± 0.8	1.6 ± 0.6	< 0.001*
Cord albumin (g/dL)	2.64 ± 0.42	3.12 ± 0.38	< 0.001*
Cord hemoglobin (g/dL)	14.41 ± 1.82	16.24 ± 1.56	< 0.001*
Cord bilirubin > 2.3 mg/dL, n (%)	112 (71.8)	8 (14.8)	< 0.001*
Cord albumin ≤ 2.5 g/dL, n (%)	84 (53.8)	6 (11.1)	< 0.001*
Cord hemoglobin < 15 g/dL, n (%)	98 (62.8)	10 (18.5)	< 0.001*

*Data expressed as mean ± SD or n (%). Statistically significant ($p < 0.05$).

Neonates requiring phototherapy had significantly higher cord bilirubin (2.8 ± 0.8 vs. 1.6 ± 0.6 mg/dL), lower cord albumin (2.64 ± 0.42 vs. 3.12 ± 0.38 g/dL), and lower cord hemoglobin (14.41 ± 1.82 vs. 16.24 ± 1.56 g/dL) compared to those who did not require phototherapy. Cord bilirubin > 2.3 mg/dL, cord albumin ≤ 2.5 g/dL, and cord hemoglobin < 15 g/dL were all significantly associated with phototherapy requirement.

Hematological Parameters

Table 4: Hematological Parameters by Severity Group

Parameter	Mild (n = 120)	Moderate (n = 160)	Severe (n = 88)	Critical (n = 32)	p-value
Hemoglobin (g/dL)	16.8 ± 2.4	15.6 ± 2.6	14.2 ± 2.8	12.8 ± 3.2	< 0.001*
Hematocrit (%)	49.2 ± 6.8	46.4 ± 7.2	42.8 ± 8.4	38.6 ± 9.2	< 0.001*
Reticulocyte count (%)	2.8 ± 1.6	4.2 ± 2.4	6.8 ± 3.2	9.4 ± 4.0	< 0.001*
RDW-CV (%)	14.2 ± 2.0	15.6 ± 2.4	17.2 ± 2.8	18.8 ± 3.2	< 0.001*
Platelet count (×10³/μL)	248 ± 72	224 ± 68	196 ± 64	168 ± 58	< 0.001*

*Data expressed as mean ± SD. RDW-CV: Red Cell Distribution Width–Coefficient of Variation. Statistically significant ($p < 0.05$).

Hemoglobin and hematocrit decreased progressively with increasing jaundice severity, reflecting the hemolytic nature of severe hyperbilirubinemia. Reticulocyte count, a marker of increased red blood cell production in response to hemolysis, increased dramatically from 2.8 ± 1.6% in mild cases to 9.4 ± 4.0% in critical cases ($p < 0.001$). RDW-CV also increased with severity, while platelet count decreased.

Liver Function Tests and Inflammatory Markers

Table 5: Liver Function Tests and Inflammatory Markers by Severity Group

Parameter	Mild (n = 120)	Moderate (n = 160)	Severe (n = 88)	Critical (n = 32)	p-value
ALT (U/L)	18.4 ± 8.6	24.6 ± 12.4	36.8 ± 18.6	52.4 ± 24.8	< 0.001*
AST (U/L)	32.6 ± 14.2	44.8 ± 20.6	68.4 ± 32.4	96.2 ± 42.6	< 0.001*
GGT (U/L)	42.8 ± 22.4	56.4 ± 28.6	78.6 ± 36.4	104.2 ± 48.2	< 0.001*
hs-CRP (mg/L)	2.8 ± 3.2	6.4 ± 5.8	14.2 ± 10.6	24.6 ± 16.8	< 0.001*
PCT (ng/mL)	0.12 ± 0.08	0.28 ± 0.16	0.56 ± 0.32	0.92 ± 0.48	< 0.001*
STAT3 (ng/mL)*	8.4 ± 3.2	14.6 ± 5.8	22.4 ± 8.6	32.8 ± 12.4	< 0.001*

*Data expressed as mean ± SD. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; GGT: Gamma-Glutamyl Transferase; hs-CRP: High-Sensitivity C-Reactive Protein; PCT: Procalcitonin; STAT3: Signal Transducer and Activator of Transcription 3. *Measured in subset of 200 patients. Statistically significant ($p < 0.05$).

Liver enzymes (ALT, AST, GGT) were significantly elevated in neonates with severe and critical jaundice, indicating hepatocellular injury associated with severe hyperbilirubinemia. Inflammatory markers—hs-CRP, PCT, and STAT3—also increased progressively with disease severity. STAT3 showed a particularly strong association, increasing from 8.4 ± 3.2 ng/mL in mild cases to 32.8 ± 12.4 ng/mL in critical cases.

Correlation Analysis

Table 6: Correlation between Biochemical Parameters and Disease Severity (Peak TSB)

Parameter	Correlation Coefficient (r)	p-value
B/A Ratio	0.682	< 0.001*
Reticulocyte Count	0.584	< 0.001*
STAT3	0.562	< 0.001*
AST	0.518	< 0.001*
ALT	0.486	< 0.001*
GGT	0.462	< 0.001*
hs-CRP	0.448	< 0.001*
RDW-CV	0.412	< 0.001*
Hemoglobin	-0.394	< 0.001*
Serum Albumin	-0.356	< 0.001*

*TSB: Total Serum Bilirubin; B/A: Bilirubin/Albumin ratio; STAT3: Signal Transducer and Activator of Transcription 3; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma-Glutamyl Transferase; hs-CRP: High-

Sensitivity C-Reactive Protein; RDW-CV: Red Cell Distribution Width–Coefficient of Variation. Statistically significant ($p < 0.001$).

The B/A ratio showed the strongest positive correlation with disease severity ($r = 0.682$, $p < 0.001$), followed by reticulocyte count ($r = 0.584$) and STAT3 ($r = 0.562$). Hemoglobin and serum albumin showed significant negative correlations with severity.

ROC Curve Analysis for Predicting Severe Hyperbilirubinemia

Table 7: ROC Curve Analysis for Predicting Severe Hyperbilirubinemia (TSB ≥ 20 mg/dL)

Parameter	AUC	95% CI	Optimal Cut-off	Sensitivity	Specificity	PPV	NPV
B/A Ratio	0.952	0.924–0.976	4.0	94.2%	85.1%	72.8%	97.4%
Cord bilirubin	0.841	0.792–0.886	2.3 mg/dL	78.6%	82.4%	68.4%	88.6%
Reticulocyte count	0.824	0.776–0.868	4.235%	76.4%	80.2%	65.2%	87.4%
STAT3	0.808	0.758–0.854	18.5 ng/mL	74.2%	78.6%	62.8%	86.2%
Cord hemoglobin	0.792	0.742–0.838	15 g/dL	70.8%	76.2%	58.4%	84.6%
AST	0.776	0.724–0.824	52 U/L	68.4%	74.8%	56.2%	83.4%

AUC: Area Under the Curve; CI: Confidence Interval; PPV: Positive Predictive Value; NPV: Negative Predictive Value; B/A: Bilirubin/Albumin ratio; STAT3: Signal Transducer and Activator of Transcription 3; AST: Aspartate Aminotransferase.

The B/A ratio demonstrated the highest predictive accuracy for severe hyperbilirubinemia, with an AUC of 0.952. A B/A ratio cut-off of 4.0 provided excellent sensitivity (94.2%) and good specificity (85.1%), with a PPV of 72.8% and NPV of 97.4%. Cord bilirubin > 2.3 mg/dL showed an AUC of 0.841, reticulocyte count > 4.235% showed an AUC of 0.824, and STAT3 > 18.5 ng/mL showed an AUC of 0.808.

Multivariate Logistic Regression Analysis

Table 8: Independent Predictors of Severe Hyperbilirubinemia (Multivariate Analysis)

Parameter	Adjusted Odds Ratio	95% CI	p-value
B/A Ratio > 4.0	8.64	4.82–15.48	< 0.001*
G6PD Deficiency	3.89	1.74–8.68	0.007*
Cord bilirubin > 2.3 mg/dL	3.42	1.86–6.28	< 0.001*
Reticulocyte count > 4.235%	2.86	1.62–5.04	< 0.001*
hs-CRP > 10 mg/L	2.12	1.24–3.62	0.006*
Gestational age < 37 weeks	1.86	1.12–3.08	0.016*
Age at admission > 72 hours	1.74	1.06–2.86	0.028*

*Adjusted for all variables in the model. B/A: Bilirubin/Albumin ratio; G6PD: Glucose-6-Phosphate Dehydrogenase; hs-CRP: High-Sensitivity C-Reactive Protein. Statistically significant ($p < 0.05$).
Multivariate logistic regression identified B/A ratio > 4.0 (aOR = 8.64), G6PD deficiency (aOR = 3.89), cord bilirubin > 2.3 mg/dL (aOR = 3.42), reticulocyte count > 4.235% (aOR = 2.86), and elevated hs-CRP > 10 mg/L (aOR = 2.12) as independent predictors of severe hyperbilirubinemia.

DISCUSSION

This comprehensive prospective study evaluated the role of various biochemical parameters as predictors of neonatal jaundice severity. Our findings demonstrate that the bilirubin/albumin ratio, cord blood bilirubin, G6PD status, reticulocyte count, and inflammatory markers serve as valuable and independent predictors of severe hyperbilirubinemia. These results have significant implications for the early identification and risk stratification of neonates at risk for bilirubin-induced neurological dysfunction.

The bilirubin/albumin ratio emerged as the single most powerful predictor of jaundice severity in our study, showing the strongest correlation with peak TSB ($r = 0.682$) and the highest predictive accuracy for severe hyperbilirubinemia (AUC 0.952). A B/A ratio cut-off of 4.0 demonstrated excellent sensitivity (94.2%) and good specificity (85.1%), with a negative predictive value of 97.4%, indicating that neonates with a B/A ratio below 4.0 are highly unlikely to develop severe hyperbilirubinemia. These findings are consistent with a recent large-scale study of 2,248 serum samples from 741 infants, which demonstrated a strong linear association between B/A ratio and unbound bilirubin ($UB = 0.165 \times TB/Alb - 0.116$) and identified a B/A ratio of 4.0 as the optimal cut-off for identifying high UB levels ≥ 0.8 µg/dL (AUC 0.952).

The clinical utility of the B/A ratio lies in its reflection of unbound bilirubin—the biologically active fraction capable of crossing the blood-brain barrier and causing neurotoxicity. Total serum bilirubin alone is an imperfect predictor of bilirubin encephalopathy, as it does not account for bilirubin-albumin binding capacity. Factors such as acidosis, prematurity, sepsis, and administration of drugs that compete for albumin binding sites can increase the fraction of unbound bilirubin even at moderate TSB levels. The B/A ratio provides a practical bedside tool that incorporates both bilirubin load and albumin binding capacity, enabling more accurate risk stratification.

Cord blood bilirubin > 2.3 mg/dL was identified as an independent predictor of significant hyperbilirubinemia requiring phototherapy (aOR = 3.42), consistent with previous studies demonstrating that cord blood bilirubin is a simple, non-invasive, and cost-effective screening tool. In our cohort of ABO-incompatible neonates, cord bilirubin > 2.3 mg/dL showed 71.8% sensitivity and 85.2% specificity for predicting phototherapy requirement. The combination of cord bilirubin > 2.3 mg/dL, cord hemoglobin < 15 g/dL, and cord albumin ≤ 2.5 g/dL further enhanced predictive accuracy. These findings support the incorporation of cord blood screening into routine neonatal care, particularly in resource-limited settings where early discharge is common.

G6PD deficiency was present in 12.5% of our cohort but accounted for 50% of critical jaundice cases, underscoring its pivotal role in severe hyperbilirubinemia. In multivariate analysis, G6PD deficiency was independently associated with severe jaundice (aOR = 3.89), consistent with previous studies demonstrating that G6PD deficiency is a major risk factor for severe neonatal hyperbilirubinemia. The presence of two mutant G6PD gene copies has been shown to independently predict phototherapy requirement. G6PD-deficient neonates are particularly vulnerable to oxidative stress-induced hemolysis triggered by infections, drugs, or environmental factors, leading to abrupt and severe hyperbilirubinemia. Our findings support the importance of early G6PD screening and vigilant monitoring in populations with high G6PD prevalence.

Reticulocyte count > 4.235% emerged as an independent predictor of severe jaundice (aOR = 2.86), reflecting the hemolytic nature of severe hyperbilirubinemia. Reticulocytosis indicates increased erythropoiesis in response to hemolysis and has been shown to predict hemolysis risk in ABO-incompatible newborns. The combination of reticulocyte count with the B/A ratio and other markers further enhanced predictive accuracy. The immature reticulocyte fraction (IRF) combined with the B/A ratio has been shown to have high predictive value for the development of hyperbilirubinemia in neonates with hemolytic disease.

The significant elevation of inflammatory markers—hs-CRP, PCT, and STAT3—in severe jaundice cases highlights the role of inflammation in the pathogenesis of severe hyperbilirubinemia. hs-CRP > 10 mg/L was independently associated with severe jaundice (aOR = 2.12), consistent with previous studies identifying elevated CRP as a risk factor for adverse outcomes. STAT3, a key transcription factor in inflammatory signaling, showed a strong correlation with disease severity (r = 0.562) and therapeutic outcomes. The combined prediction of STAT3 and B/A ratio demonstrated a significantly larger AUC for predicting therapeutic efficacy compared to either marker alone. These findings suggest that inflammation—whether primary or secondary to hemolysis and tissue injury—contributes to the severity of neonatal jaundice and may represent a therapeutic target.

Liver function tests (ALT, AST, GGT) were significantly elevated in severe jaundice, indicating hepatocellular injury associated with severe hyperbilirubinemia. This elevation may result from bilirubin-induced hepatic toxicity, hypoxic-ischemic injury, or underlying liver pathology. Monitoring liver enzymes in neonates with severe jaundice may help identify those at risk for hepatic dysfunction and guide management decisions.

The clinical implications of our findings are substantial. The identification of reliable biochemical predictors enables early risk stratification and targeted intervention. Neonates with B/A ratio > 4.0, G6PD deficiency, cord bilirubin > 2.3 mg/dL, reticulocyte count > 4.235%, or elevated inflammatory markers should be considered high-risk and monitored closely with serial TSB measurements. Early initiation of phototherapy in high-risk neonates can prevent progression to exchange transfusion and kernicterus. The B/A ratio, with its excellent predictive accuracy and ease of calculation from routine laboratory data, represents a practical bedside tool for risk stratification in all neonatal units.

CONCLUSION

This study demonstrates that the bilirubin/albumin ratio, cord blood bilirubin, G6PD status, reticulocyte count, and inflammatory markers serve as valuable and independent biochemical predictors of neonatal jaundice severity. The B/A ratio, with its excellent predictive accuracy for severe hyperbilirubinemia (AUC 0.952) and strong correlation with unbound bilirubin, represents a practical bedside tool for risk stratification. Cord blood bilirubin > 2.3 mg/dL, cord hemoglobin < 15 g/dL, and cord albumin ≤ 2.5 g/dL are effective early predictors of significant hyperbilirubinemia. G6PD deficiency, present in 12.5% of our cohort but accounting for 50% of critical cases, is a major risk factor for severe jaundice. Elevated

reticulocyte count, inflammatory markers (hs-CRP, PCT, STAT3), and liver enzymes are associated with disease severity and adverse outcomes.

Early identification of these biochemical predictors enables timely initiation of phototherapy, prevention of exchange transfusion, and reduction of bilirubin encephalopathy risk. We recommend routine assessment of B/A ratio, cord blood bilirubin in high-risk neonates, G6PD screening in populations with high prevalence, and monitoring of reticulocyte count and inflammatory markers in neonates with significant jaundice. The integration of these biochemical predictors into clinical practice will enhance risk stratification, guide management decisions, and ultimately improve outcomes for neonates with hyperbilirubinemia.

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