RESEARCH PAPER

CORD BLOOD BILIRUBIN AS AN EARLY PREDICTOR OF NEONATAL HYPERBILIRUBINEMIA IN NEONATES WITH ABO AND Rh INCOMPATIBILITY

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ABSTRACT

Background: Neonatal hyperbilirubinemia is a common clinical condition in the early neonatal period and remains a significant cause of neonatal morbidity, particularly in newborns with ABO and Rh incompatibility. Early identification of neonates at risk of developing clinically significant hyperbilirubinemia is essential to enable timely intervention, support safe early postpartum discharge, and prevent severe outcomes such as exchange transfusion. Cord blood bilirubin has been proposed as a simple and early predictive marker for subsequent hyperbilirubinemia.

Objectives: To evaluate the usefulness of cord blood bilirubin in predicting subsequent hyperbilirubinemia in neonates with ABO and Rh incompatibility, and to assess its role in identifying newborns requiring therapeutic intervention, thereby facilitating early postpartum discharge and reducing the need for exchange transfusion.

Methods: This prospective observational study included 142 mother–newborn pairs with ABO and/or Rh incompatibility. Maternal demographic data, obstetric details, and neonatal baseline characteristics were recorded. Cord blood total serum bilirubin was measured at birth. Neonates were followed clinically and biochemically for the development of visible jaundice, serum bilirubin levels at 48 and 72 hours, need for phototherapy, exchange transfusion, duration of hospital stay, and overall outcome. Statistical analysis included comparison of mean cord bilirubin levels between outcome groups and receiver operating characteristic (ROC) curve analysis to determine predictive accuracy and optimal cutoff values.

Results: The mean cord blood total serum bilirubin level was 3.16 ± 0.82 mg/dL. Visible jaundice developed in 47.2% of neonates, while 14.8% required phototherapy and only 2.1% required exchange transfusion. Neonates who developed visible jaundice had significantly higher mean cord bilirubin levels (3.75 ± 0.54 mg/dL) compared to those without jaundice (2.62 ± 0.65 mg/dL; $p < 0.001$). Similarly, neonates requiring phototherapy had significantly higher cord bilirubin levels (3.81 ± 0.55 mg/dL) than those who did not require treatment (3.04 ± 0.81 mg/dL; $p < 0.001$). Although cord bilirubin levels were higher in neonates who required exchange transfusion, this association was not statistically significant due to the small number of severe cases. ROC curve analysis demonstrated good predictive accuracy of cord bilirubin for phototherapy requirement with an area under the curve of 0.794 (95% CI: 0.711–0.876). A cord bilirubin cutoff value of 3.27 mg/dL provided high sensitivity (90.5%) and moderate specificity (66.9%) for predicting the need for phototherapy.

Conclusion: Cord blood bilirubin is a useful early predictor of subsequent clinically significant hyperbilirubinemia in neonates with ABO and Rh incompatibility. Higher cord bilirubin levels are strongly associated with the development of visible jaundice and the need for phototherapy. Although its ability to predict severe outcomes such as exchange transfusion is limited by the low incidence of such events, cord bilirubin estimation at birth can serve as an effective screening tool to guide early risk stratification, support safe early postpartum discharge, and enable timely therapeutic intervention.

Keywords: Cord blood bilirubin, Neonatal hyperbilirubinemia, ABO incompatibility, Rh incompatibility, Phototherapy, Exchange transfusion, Early discharge

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INTRODUCTION

Neonatal hyperbilirubinemia (NNHB) is one of the most frequently encountered clinical conditions in the neonatal period, affecting approximately 60% of term and 80% of preterm infants during the first week of life. It is characterized by yellowish discoloration of the skin and sclera resulting from excessive accumulation of bilirubin in body tissues.1 Nearly all neonates experience a transient increase in unconjugated serum bilirubin levels exceeding 30 mmol/L (1.8 mg/dL) during this period.2 Although the majority of cases are benign and self-limiting, early identification and monitoring of infants at risk of developing severe hyperbilirubinemia are essential, as elevated bilirubin levels can lead to kernicterus and irreversible neurological injury if untreated.3

The physiological rise in bilirubin is primarily attributed to the breakdown of fetal haemoglobin, immature hepatic conjugation mechanisms, and enhanced enterohepatic circulation.4,5 Typically, bilirubin concentrations peak between the fifth and seventh days of life and subsequently decline. However, in pathological conditions—either congenital or acquired—bilirubin accumulation can become excessive. Because unconjugated bilirubin is lipid-soluble, it can cross the blood–brain barrier, leading to bilirubin-induced neurologic dysfunction (BIND).6

Neonatal hyperbilirubinemia is broadly classified into unconjugated (indirect) and conjugated (direct) forms. The unconjugated variant is more common and may be physiological or pathological. Physiologic jaundice generally appears after 24 hours of life, peaks within 48–96 hours, and resolves within two to three weeks in term neonates.7 Conversely, pathologic unconjugated hyperbilirubinemia (UHB) presents within the first 24 hours, with total serum bilirubin (TSB) exceeding the 95th percentile for age, or rising by ≥5 mg/dL per day or >0.2 mg/dL per hour.2

The American Academy of Paediatrics (AAP) guidelines recommend the universal screening of all neonates before discharge to identify those at risk for severe unconjugated hyperbilirubinemia and bilirubin encephalopathy.2 Follow-up evaluation within 48 to 72 hours post-discharge is advised for neonates released earlier than three days of life.8 Despite these recommendations, NNHB continues to contribute significantly to neonatal morbidity and mortality, particularly in low- and middle-income countries.9 While the incidence of neonatal jaundice is estimated at 60–70% in Western populations,10 it is notably higher among Asian newborns.

Several strategies have been implemented to predict and prevent severe hyperbilirubinemia, including early follow-up visits, pre-discharge measurement of serum or transcutaneous bilirubin, and evaluation of risk factors.11 However, early postnatal readmission due to jaundice remains common,12 imposing additional financial and emotional burdens on families, disrupting breastfeeding, and increasing hospital-acquired infection risk.13

Among various early diagnostic markers, umbilical cord blood bilirubin (CBB) has emerged as a promising, non-invasive, and cost-effective predictor for the development of significant hyperbilirubinemia. This marker provides an immediate postnatal biochemical snapshot reflecting intrauterine bilirubin production and potential hemolytic activity. Notably, ABO and Rh incompatibility are recognized as major etiological factors for hemolytic disease of the newborn, and CBB measurement offers valuable insights into these conditions.14,15

Numerous studies have demonstrated that neonates with elevated CBB levels are significantly more likely to develop hyperbilirubinemia requiring phototherapy. Karthikeyan Panneerselvam et al.16 (2018) and Pradhan et al.17 (2017) reported that cord bilirubin levels above 2.25 mg/dL were strongly associated with pathological jaundice in ABO-incompatible neonates. Zeitoun et al.18 (2013) further supported these findings, showing that cord bilirubin levels above 2.15 mg/dL in full-term and late preterm infants were significantly linked to the need for phototherapy.

Particularly in immune-mediated hemolytic disease, CBB serves as an essential predictive tool. Jadhav et al.14 (2025) demonstrated that a cut-off of >1.9 mg/dL in Rh-positive neonates born to Rh-negative mothers yielded excellent specificity (97.8%) and a high positive predictive value (95.74%) for identifying infants requiring treatment. Likewise, Krishnan et al.15 (2016) found that cord bilirubin levels exceeding 1.8 mg/dL in ABO-incompatible newborns were predictive of pathological hyperbilirubinemia, underscoring the biomarker's diagnostic value in immunohematological incompatibility.

Neonatal hyperbilirubinemia continues to be a major cause of morbidity in the early neonatal period, particularly among babies with ABO and Rh incompatibility, where hemolysis leads to excessive bilirubin production. Despite the availability of screening guidelines and postnatal follow-up recommendations, early discharge practices often result in delayed detection of significant jaundice, increasing the risk of bilirubin encephalopathy and kernicterus. There is, therefore, a pressing need for a simple, cost-effective, and non-invasive method that can identify high-risk neonates at birth, allowing for timely monitoring and early therapeutic intervention. Measurement of umbilical cord blood bilirubin (CBB) immediately after delivery offers such an opportunity—it reflects the infant's intrauterine bilirubin load and provides early insight into the risk of hemolytic hyperbilirubinemia before clinical signs appear. In view of this, the present study was undertaken.

OBJECTIVES OF THE STUDY

The main objectives of the study are:

To predict the usefulness of cord blood bilirubin in identifying subsequent hyperbilirubinemia in ABO and Rh incompatible babies requiring therapeutic intervention, so that early postpartum discharge of both mother and newborns can be planned.

To reduce the rate of babies requiring exchange transfusion by enabling early identification and timely therapeutic intervention through cord blood bilirubin screening.

MATERIALS AND METHODS

Study Design and Setting

This hospital-based, prospective observational study was conducted in the Department of Paediatrics at Adichunchanagiri Institute of Medical Sciences (AIMS), B.G. Nagara, Mandya District, Karnataka, from May 2024 to December 2025. The study population comprised live-born neonates delivered at the

institute during the study period to mothers with O+ or Rh-negative blood group.

Sample Size

The sample size was calculated using OpenEpi Version 3.0 software, based on the data from the study by C. Baskar et al., with a confidence interval of 95%, power of 80%, and a standard deviation of the reference population. The estimated minimum required sample size was 142 neonates. A convenient sampling technique was employed, and all eligible neonates meeting the inclusion criteria during the study period were consecutively enrolled until the required sample size was achieved.

Inclusion Criteria

Newborns born to O+ or Rh-negative blood group mothers.

Newborns with gestational age (GA) > 37 weeks.

Newborns with birth weight between 2.5–4 kg.

Newborns with APGAR score > 7 at 5 minutes.

Exclusion Criteria

Neonates with significant illness or major congenital malformations.

Neonates with conditions known to affect bilirubin levels independently, such as sepsis, hypothyroidism, or respiratory distress syndrome.

Neonates with traumatic birth injuries such as cephalhematoma.

Newborns from multiple pregnancies.

Newborns with uncertain gestational age, defined as a discrepancy of more than two weeks between obstetric and clinical gestational age assessment.

Data Collection and Procedure

The study protocol was approved by the Institutional Ethics Committee of Adichunchanagiri Institute of Medical Sciences, and written informed consent was obtained from the parents or guardians of all participants prior to enrollment. All procedures were conducted in accordance with the Declaration of Helsinki.

After delivery, umbilical cord blood samples (approximately 2–3 mL) were collected from the placental end of the umbilical vein under aseptic precautions immediately after cord clamping. The samples were analyzed in the hospital's biochemistry laboratory for: (i) Total Serum Bilirubin (TSB) estimation using the Diazo method; (ii) Blood grouping and Rh typing of both mother and neonate; and (iii) Direct Coombs Test (DCT) to detect immune-mediated hemolysis where applicable.

All enrolled neonates were clinically examined daily for five consecutive days or until discharge. Any infant showing visible jaundice was assessed for serum bilirubin at 48 and 72 hours post-delivery. The American Academy of Pediatrics (AAP) 2004 phototherapy nomogram was used to determine the need for phototherapy. Neonates requiring phototherapy were managed according to institutional neonatal jaundice protocols. Relevant maternal details including age, parity, and obstetric history were recorded, along with neonatal parameters including birth weight, gestational age, APGAR scores, and mode of delivery.

Statistical Analysis

All collected data were entered and processed using Microsoft Excel and SPSS Version 25.0. Descriptive statistics including mean, standard deviation, and percentage distribution were calculated for all variables. The association between cord blood bilirubin levels and the development of significant hyperbilirubinemia was analyzed using the Chi-Square test and independent samples t-test as appropriate. Effect sizes were calculated using eta-squared (η^2). Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of cord bilirubin in predicting the need for phototherapy, and the optimal cutoff value was determined using Youden's Index. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed at the optimal cutoff. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 142 mother–newborn pairs meeting the inclusion criteria were enrolled in the study. The demographic, clinical, and biochemical findings are presented in the following tables.

Table 1. Summary of Baseline Characteristics

Parameter	Value
Total enrolled neonates	142
Mean gestational age (weeks)	39.17 ± 1.48
Mean birth weight (kg)	3.04 ± 0.42
Mean APGAR score at 1 min	7.99 ± 0.81
Mean APGAR score at 5 min	8.48 ± 0.92
Sex: Female/Male	74 (52.1%) / 68 (47.9%)
Mean cord blood TSB (mg/dL)	3.16 ± 0.82
ABO/Rh incompatibility	126 (88.7%)
DCT Positive	43 (30.3%)
Visible jaundice	67 (47.2%)
Phototherapy required	21 (14.8%)
Exchange transfusion	3 (2.1%)
Healthy outcome	139 (97.9%)

TSB = Total Serum Bilirubin; DCT = Direct Coombs Test

Table 2. Maternal Age Distribution

Age Group (Years)	Frequency	Percentage (%)
20–24	47	33.1
25–29	24	16.9
30–34	39	27.5
35–39	32	22.5
Total	142	100

Among the 142 mothers, the largest proportion fell in the 20–24 year age group (47 mothers, 33.1%), followed by 30–34 years (39 mothers, 27.5%), and 35–39 years (32 mothers, 22.5%). The 25–29 year group contributed the smallest share with 24 mothers (16.9%). The distribution indicates that most participants were in their early twenties to early thirties, with substantial representation even in the 35–39 year category, indicating the study population was not limited to younger mothers alone.

Table 3. Parity Distribution

Parity Group	Frequency	Percentage (%)
G1P0	32	22.5
G2P1	25	17.6
G3P2	29	20.4
G4P3	26	18.3
G5P4	30	21.1
Total	142	100

Parity was fairly evenly distributed across groups. Primigravida mothers (G1P0) accounted for 32 cases (22.5%). G2P1 represented 25 mothers (17.6%), while G3P2 included 29 mothers (20.4%). G4P3 comprised 26 mothers (18.3%) and G5P4 made up 30 mothers (21.1%). This balanced parity distribution reduces the chance that results are driven only by primiparity or high parity.

Table 4. Gestational Age Distribution

Gestational Age (weeks)	Frequency	Percentage (%)
37	26	18.3
38	28	19.7
39	23	16.2
40	26	18.3
41	39	27.5
Total	142	100
Mean ± SD	39.17 ± 1.483 weeks	

Gestational age ranged from 37 to 41 weeks, with the highest frequency at 41 weeks (39 neonates, 27.5%). The mean gestational age was 39.17 ± 1.483 weeks, reflecting a predominantly term population with moderate variability .

Table 5. Maternal Blood Group Distribution

Blood Group	Frequency	Percentage (%)
A	35	24.6
AB	31	21.8
B	45	31.7
O	31	21.8
Total	142	100

Maternal blood group B was the most common, occurring in 45 mothers (31.7%). Group A was present in 35 mothers (24.6%), while AB and O were each recorded in 31 mothers (21.8% each). The higher prevalence of blood group B in the maternal cohort is relevant to ABO incompatibility patterns and hemolysis-related hyperbilirubinemia risk.

Table 6. Maternal Rh Status

Maternal Rh	Frequency	Percentage (%)
Negative	75	52.8
Positive	67	47.2
Total	142	100

Rh-negative mothers formed a slight majority with 75 cases (52.8%), compared with 67 Rh-positive mothers (47.2%). The relatively high proportion of Rh-negative mothers increases the potential pool for Rh incompatibility scenarios when paired with Rh-positive neonates.

Table 7. Neonatal Rh Status

Neonatal Rh	Frequency	Percentage (%)
Negative	82	57.7
Positive	60	42.3
Total	142	100

Neonatal Rh status showed 82 neonates (57.7%) were Rh-negative, while 60 (42.3%) were Rh-positive. The 42.3% Rh-positive neonates provide a meaningful subgroup where Rh-related hemolytic risk may occur, particularly when born to Rh-negative mothers.

Table 8. ABO and Rh Incompatibility Status

ABO/Rh Incompatibility	Frequency	Percentage (%)
Yes	126	88.7
No	16	11.3
Total	142	100

A very large majority of neonates were classified as having ABO/Rh incompatibility, with 126 cases (88.7%), while only 16 cases (11.3%) had no incompatibility. This high prevalence of incompatibility makes the cohort particularly relevant for evaluating cord bilirubin as a predictive marker.

Table 9. Direct Coombs Test (DCT) Result

DCT Result	Frequency	Percentage (%)
Positive	43	30.3
Negative	99	69.7
Total	142	100

The Direct Coombs Test (DCT) was positive in 43 neonates (30.3%) and negative in 99 neonates (69.7%). Despite a high rate of ABO/Rh incompatibility classification, only about one-third showed evidence of antibody-mediated hemolysis detectable by DCT, suggesting that not all incompatibility cases lead to significant immune-mediated hemolysis.

Table 10. Neonatal Sex Distribution

Sex	Frequency	Percentage (%)
Female	74	52.1
Male	68	47.9
Total	142	100

The neonatal sex distribution was near equal, with a slight female predominance: 74 females (52.1%) and 68 males (47.9%). This balanced distribution reduces the likelihood that observed bilirubin outcomes are confounded by significant sex imbalance within the cohort.

Table 11. Neonatal Blood Group Distribution

Neonatal Blood Group	Frequency	Percentage (%)
A	37	26.1
AB	44	31.0
B	28	19.7
O	33	23.2
Total	142	100

Neonatal blood group AB was the most frequent with 44 neonates (31.0%), followed by A with 37 (26.1%), O with 33 (23.2%), and B with 28 (19.7%). The distribution supports the study's emphasis on incompatibility-related jaundice risk assessment.

Table 12. Mean Cord Blood Total Serum Bilirubin (TSB)

Parameter	Mean	Standard Deviation
Cord TSB (mg/dL)	3.1551	0.82381

The mean cord TSB was 3.16 ± 0.82 mg/dL, reflecting a wide distribution consistent with the heterogeneous nature of the study population with varying degrees of incompatibility and hemolytic activity.

Table 13. Birth Weight Categories

Weight Category (kg)	Frequency	Percentage (%)
< 2.5	17	12.0
2.5 – 2.9	47	33.1
3.0 – 3.4	52	36.6
3.5 – 3.9	20	14.1
≥ 4.0	6	4.2
Total	142	100
Mean ± SD	3.04 ± 0.42 kg	

Birth weight was most commonly in the 3.0–3.4 kg group (52 neonates, 36.6%), followed by 2.5–2.9 kg (47 neonates, 33.1%). The mean birth weight was 3.04 ± 0.42 kg, suggesting most babies were of average term weight.

Table 14. APGAR Score at 1 Minute

APGAR Score (1 min)	Frequency	Percentage (%)
7	47	33.1
8	49	34.5
9	46	32.4
Total	142	100
Mean ± SD	7.99 ± 0.812	

Table 15. APGAR Score at 5 Minutes

APGAR Score (5 min)	Frequency	Percentage (%)
7	22	15.5
8	50	35.2
9	50	35.2
10	20	14.1
Total	142	100
Mean ± SD	8.48 ± 0.92	

APGAR scores at 1 minute ranged from 7 to 9 (mean 7.99 ± 0.81), and at 5 minutes ranged from 7 to 10 (mean 8.48 ± 0.92), indicating good immediate neonatal adaptation at birth across the cohort with minimal confounding from perinatal distress.

Table 16. Mode of Delivery

Mode of Delivery	Frequency	Percentage (%)
Cesarean	81	57.0
Normal Vaginal	61	43.0
Total	142	100

Cesarean delivery was more common than normal vaginal delivery in this cohort, with 81 cesarean births (57%) compared to 61 vaginal births (43%), reflecting institutional delivery patterns.

Table 17. Visible Jaundice

Visible Jaundice	Frequency	Percentage (%)
Yes	67	47.2
No	75	52.8
Total	142	100

Visible jaundice was present in 67 neonates (47.2%) and absent in 75 neonates (52.8%). Nearly half of the babies developed clinically noticeable jaundice, emphasizing the high burden of hyperbilirubinemia in this incompatibility-enriched cohort.

Table 18. Postnatal Serum Bilirubin Levels

Parameter	Mean (mg/dL)	Standard Deviation
TSB at 48 hours	7.02	3.08
TSB at 72 hours	6.70	2.20

Mean TSB at 48 hours was 7.02 ± 3.08 mg/dL, while at 72 hours it was 6.70 ± 2.20 mg/dL. The large standard deviation at 48 hours indicates wide variability in bilirubin rise among neonates, consistent with a mix of mild and high-risk cases.

Table 19. Phototherapy Requirement

Phototherapy Required	Frequency	Percentage (%)
Yes	21	14.8
No	121	85.2
Total	142	100

Phototherapy was required in 21 neonates (14.8%), while 121 (85.2%) did not require it. Although incompatibility was very common in the cohort, only a minority progressed to clinically significant hyperbilirubinemia warranting phototherapy.

Table 20. Exchange Transfusion

Exchange Transfusion	Frequency	Percentage (%)
Yes	3	2.1
No	139	97.9
Total	142	100

Only 3 neonates (2.1%) underwent exchange transfusion. This very low proportion indicates that severe hyperbilirubinemia requiring exchange transfusion was uncommon in the cohort, likely reflecting effective monitoring, timely phototherapy, and a generally limited severity of hemolysis in most babies.

Table 21. Duration of Hospital Stay

Hospital Stay (days)	Frequency	Percentage (%)
2	35	24.6
3	37	26.1
4	36	25.4
5	34	23.9
Total	142	100
Mean ± SD	3.49 ± 1.109 days	

Hospital stay ranged from 2 to 5 days, with 3 days being the most frequent (26.1%). The mean stay was 3.49 ± 1.109 days, indicating moderate variability in clinical course

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Table 22. Clinical Outcome

Outcome	Frequency	Percentage (%)
Healthy	139	97.9
Complications	3	2.1
Total	142	100

The vast majority of neonates were discharged as healthy (139 cases, 97.9%), while 3 neonates (2.1%) had complications. These favorable outcomes suggest effective clinical management, though the small subset with complications highlights the importance of early predictive markers.

Table 23. Mean Cord TSB vs Visible Jaundice

Visible Jaundice	Mean ± SD (mg/dL)	p-value
Yes	3.75 ± 0.54	< 0.001
No	2.62 ± 0.65	

Effect size: $\eta^2 = 0.474$ (large)

Neonates who developed visible jaundice had a markedly higher mean cord TSB (3.75 ± 0.54 mg/dL) compared with those without visible jaundice (2.62 ± 0.65 mg/dL; $p < 0.001$). The large effect size ($\eta^2 = 0.474$) indicates that a substantial proportion of the variance in cord bilirubin is explained by subsequent jaundice visibility, strongly supporting cord TSB as an early predictor.

Table 24. Mean Cord TSB vs Phototherapy Requirement

Phototherapy Required	Mean ± SD (mg/dL)	p-value
Yes	3.81 ± 0.55	< 0.001
No	3.04 ± 0.81	

Effect size: $\eta^2 = 0.109$ (moderate)

Babies who required phototherapy had significantly higher mean cord TSB (3.81 ± 0.55 mg/dL) compared with those who did not (3.04 ± 0.81 mg/dL; $p < 0.001$). The moderate effect size ($\eta^2 = 0.109$) confirms that cord TSB contributes meaningfully to predicting phototherapy need.

Table 25. Mean Cord TSB vs Exchange Transfusion

Exchange Transfusion	Mean ± SD (mg/dL)	p-value
Yes	3.73 ± 0.40	0.22
No	3.14 ± 0.83	

Effect size: $\eta^2 = 0.011$ (small). Non-significant due to low event count (n=3).

Mean cord TSB was higher in the exchange transfusion group (3.73 ± 0.40 mg/dL) compared with the non-exchange group (3.14 ± 0.83 mg/dL), but this difference was not statistically significant ($p = 0.22$). The small effect size ($\eta^2 = 0.011$) and very limited event count (n=3) preclude definitive conclusions regarding prediction of exchange transfusion.

Table 26. Mean Cord TSB vs Clinical Outcome

Outcome	Mean ± SD (mg/dL)	p-value
Healthy	3.14 ± 0.83	0.22
Complications	3.73 ± 0.40	

Cord TSB levels in neonates with complications averaged 3.73 ± 0.40 mg/dL versus 3.14 ± 0.83 mg/dL in those with healthy outcomes, but this difference was not statistically significant ($p = 0.22$), likely due to the small number of complication cases (n = 3).

Table 27. ROC Curve Analysis – Cord TSB for Phototherapy Prediction

Metric	Value
Area Under the Curve (AUC)	0.794
Standard Error	0.042
95% Confidence Interval	0.711 – 0.876
Asymptotic Significance (p-value)	< 0.001

The ROC analysis demonstrated an AUC of 0.794 (95% CI: 0.711–0.876; $p < 0.001$), indicating good predictive ability of cord TSB to distinguish between neonates who will require phototherapy and those who will not. This is clearly superior to chance (AUC 0.5) and supports cord bilirubin as a clinically useful screening measure.

Table 28. Optimal Cutoff Value Based on Youden's Index

Optimal Cutoff	Sensitivity	Specificity	Youden's Index
3.27 mg/dL	90.5%	66.9%	0.574

The optimal cutoff for cord TSB was identified as 3.27 mg/dL. At this threshold, sensitivity was 90.5% (correctly identifying about 9 out of 10 neonates who will require phototherapy), and specificity was 66.9%. The Youden's Index of 0.574 reflects a reasonable balance between sensitivity and specificity, supporting the practical utility of 3.27 mg/dL as a screening cut-point in ABO/Rh incompatible newborns.

DISCUSSION

Neonatal hyperbilirubinemia remains one of the most common clinical conditions encountered in the early postnatal period and continues to be a significant cause of neonatal morbidity worldwide. Although the majority of cases are benign and self-limiting, a subset of newborns—particularly those with hemolytic risk factors such as ABO and Rh incompatibility—may develop rapidly rising bilirubin levels that require timely therapeutic intervention. Failure to identify these high-risk neonates early can result in severe complications, including bilirubin encephalopathy and kernicterus, which are largely preventable with appropriate surveillance and treatment.

In recent years, increasing emphasis has been placed on early risk stratification strategies that can predict the likelihood of subsequent significant hyperbilirubinemia soon after birth. Such strategies are especially important in the context of early postpartum discharge, where opportunities for prolonged in-hospital observation are limited. An ideal predictive marker would be simple, non-invasive, cost-effective, and available at birth, allowing clinicians to distinguish newborns who require close monitoring from those who can be safely discharged.

Cord blood bilirubin has emerged as a promising early biochemical marker in this regard. As a reflection of intrauterine bilirubin load and early hemolytic activity, cord bilirubin estimation offers the advantage of being obtained immediately at birth without additional invasive procedures. Several studies have explored its utility in predicting the development of clinically significant hyperbilirubinemia, need for phototherapy, and, in severe cases, exchange transfusion. However, reported cutoff values, predictive accuracy, and associated risk factors have varied across populations, necessitating further evaluation in different clinical settings.

Against this background, the present study was undertaken to assess the usefulness of cord blood bilirubin in predicting subsequent hyperbilirubinemia in newborns with ABO and Rh incompatibility, with the dual objectives of facilitating safe early discharge and reducing the incidence of severe hyperbilirubinemia requiring exchange transfusion. The findings are discussed below in comparison with existing literature.

Gestational Maturity and Baseline Neonatal Characteristics

In the present study, all neonates were delivered at term, with a mean gestational age of 39.17 ± 1.48 weeks and a mean birth weight of 3.04 ± 0.42 kg. The cohort demonstrated good immediate neonatal adaptation, as reflected by satisfactory APGAR scores at 1 minute (7.99 ± 0.81) and 5 minutes (8.48 ± 0.92), along with a near-equal sex distribution (52.1% females). This relatively homogeneous and clinically stable baseline minimized the confounding influence of prematurity, low birth weight, or perinatal distress on bilirubin dynamics, allowing a clearer assessment of cord bilirubin as a predictive marker.

Similar baseline characteristics have been reported across several comparable studies. Kardum D et al.74 studied a large cohort of 1360 neonates with gestational age ≥36 weeks and found no significant difference in gestational age between hyperbilirubinemic and non-hyperbilirubinemic infants (median 39 weeks in both groups). However, they noted a significantly lower mean birth weight among hyperbilirubinemic neonates (3318.6 ± 503.3 g) compared to those without hyperbilirubinemia (3414.0 ± 470.0 g), suggesting that even within term infants, relatively lower birth weight may modestly contribute to risk. Pradhan A et al.17 included 202 newborns with a mean gestational age of 38.3 ± 1.35 weeks, of whom 23.8% were late preterm (35–37 weeks). Despite this inclusion, the overall cohort remained largely stable, with a mean birth weight of 3.09 ± 0.56 kg and an almost equal female predominance (51.5%).

A predominantly term and appropriate-for-gestational-age population was also observed by Krishnan E et al.,15 who evaluated 191 term neonates at risk of ABO incompatibility, with the majority (70%) weighing between 2.5 and 3.0 kg. Hanasi S et al.77 followed 150 healthy term newborns and reported that none of the baseline variables showed a significant association with cord blood bilirubin levels, supporting the concept that cord bilirubin acts independently of basic demographic characteristics in stable term infants. Similarly, Gupta N et al.78 and Sonawane TL et al.80 found that birth weight and sex did not emerge as decisive factors influencing bilirubin outcomes in their respective cohorts. Anjanappa S et al.83 further emphasized that gestational maturity—but not sex, birth weight, or mode of delivery—was significantly associated with hyperbilirubinemia at 48 hours.

Overall, comparison across studies demonstrates consistent inclusion of predominantly term, clinically stable neonates with comparable birth weights and satisfactory APGAR scores. These similarities strengthen the validity of using cord blood bilirubin as an early predictive marker, as its association with subsequent hyperbilirubinemia appears largely independent of baseline gestational maturity, sex, and birth weight in term neonates.

Blood Group Distribution and Incompatibility Pattern

In the present study, maternal blood group B was the most common (31.7%), while neonatal blood group AB predominated (31%). More than half of the mothers were Rh-negative (52.8%), and a very high proportion of neonates (88.7%) had ABO and/or Rh incompatibility. This deliberate enrichment of incompatibility

cases created a high-risk cohort well suited for evaluating hemolysis-related neonatal hyperbilirubinemia.

Comparable observations have been reported in earlier studies, though with notable variation in incompatibility prevalence. Pradhan A et al.17 reported a much lower overall rate of blood group incompatibility (9.9%), predominantly due to ABO incompatibility (9.4%), with Rh incompatibility being rare (0.5%). Despite the lower prevalence, ABO incompatibility showed a strong and statistically significant association with pathological hyperbilirubinemia (36.8% of ABO-incompatible neonates; $p = 0.001$), supporting its role as an important risk factor even in smaller proportions. Krishnan E et al.15 found that although blood groups A positive and B positive were most common, there was no statistically significant association between neonatal blood group type and occurrence of jaundice ($p > 0.01$) in their cohort. Sonawane TL et al.80 similarly observed that blood group distribution alone did not significantly influence hyperbilirubinemia development. Taken together, these findings highlight that while blood group incompatibility remains a well-recognized risk factor, its clinical impact varies across populations, and the very high prevalence in the present study distinguishes it from many previous reports, strengthening the relevance of cord blood bilirubin estimation in this high-risk setting.

Cord Blood Bilirubin Levels

In the present study, the mean cord blood total serum bilirubin was 3.16 ± 0.82 mg/dL. This relatively higher mean compared to several other studies reflects the deliberate inclusion of a high-risk cohort with a large proportion of ABO and Rh incompatibility. Comparable but slightly lower baseline cord bilirubin values have been reported by Pradhan A et al.17 (2.47 ± 0.36 mg/dL), Hanasi S et al.77 (2.02 ± 0.36 mg/dL), and Sonawane TL et al.80 (2.60 ± 0.70 mg/dL). The relationship between cord bilirubin and later bilirubin rise was further strengthened in Gupta N et al.,78 where cord bilirubin showed a strong positive correlation with serum bilirubin at 72–96 hours ($r = 0.504$, $p = 0.001$). Collectively, these findings demonstrate consistent inter-study agreement that higher cord bilirubin values at birth reflect an increased bilirubin load and a higher likelihood of subsequent clinically significant hyperbilirubinemia.

Postnatal Bilirubin Pattern and Clinical Jaundice

In the present study, visible jaundice developed in 47.2% of neonates, with mean serum bilirubin levels of 7.02 ± 3.08 mg/dL at 48 hours and 6.70 ± 2.20 mg/dL at 72 hours, highlighting considerable variability in postnatal bilirubin trajectories even within a term population. Early bilirubin rise has been consistently documented: Kardum D et al.74 demonstrated significantly higher cord bilirubin in neonates who developed hyperbilirubinemia within the first 48 hours; Pradhan A et al.17 reported pathological hyperbilirubinemia in 12.9% of newborns; and Krishnan E et al.15 clearly illustrated that mean cord bilirubin values rose progressively from neonates without jaundice (1.35 mg/dL) to physiological jaundice (2.09 mg/dL) and pathological jaundice (3.15 mg/dL). Lower overall incidences were reported by Hanasi S et al.77 (6.6%) and Sonawane TL et al.80 (3.78%), but both still demonstrated progressive postnatal bilirubin rise. Together, these findings confirm that higher early bilirubin load consistently translates into increased risk of clinically significant jaundice.

Cord Bilirubin and Visible Jaundice

In the present study, neonates who developed visible jaundice had significantly higher mean cord bilirubin levels (3.75 ± 0.54 mg/dL) compared to those without jaundice (2.62 ± 0.65 mg/dL; $p < 0.001$; $\eta^2 = 0.474$). This relationship has been consistently demonstrated across multiple studies. Kardum D et al.74 showed that higher cord bilirubin was strongly associated with clinically significant jaundice within the first 48 hours. Pradhan A et al.17 and Krishnan E et al.15 similarly reported significantly higher cord bilirubin values among neonates with pathological jaundice. Hanasi S et al.77 found that higher cord bilirubin correlated with higher serum bilirubin at 72 hours, and Sonawane TL et al.80 showed elevated cord bilirubin predicted clinically significant jaundice within the first two days of life. Karthikeyan Panneerselvam16 reported higher mean cord bilirubin in pathological jaundice (2.6 mg/dL) compared to physiological jaundice (2.2 mg/dL). Importantly, Gupta N et al.78 highlighted the combined role of bilirubin load and binding capacity, and Jadhav DJP et al.14 showed that cord bilirubin >1.9 mg/dL was strongly associated with visible jaundice and increased need for phototherapy in high-risk hemolytic settings.

Cord Bilirubin and Phototherapy Requirement

In the present study, neonates who required phototherapy had significantly higher mean cord bilirubin levels (3.81 ± 0.55 mg/dL) compared to those who did not require treatment (3.04 ± 0.81 mg/dL; $p < 0.001$). Comparable results have been consistently reported in the literature. Pradhan A et al.17 demonstrated that a cord bilirubin cutoff of ≥2.5 mg/dL predicted pathological hyperbilirubinemia requiring phototherapy with sensitivity 84.1% and specificity 88.5%. Krishnan E et al.15 reported that a cutoff of >1.8 mg/dL identified more than 70% of neonates who later required treatment. Hanasi S et al.77 demonstrated that neonates with cord bilirubin ≥2.5 mg/dL had significantly higher serum bilirubin levels at 72 hours. Gupta N et al.78 reported a cord bilirubin cutoff of >2.33 mg/dL with sensitivity 94.12% and specificity 86.67%. Sonawane TL et al.80 observed that cord bilirubin ≥3 mg/dL predicted significant hyperbilirubinemia with 100% sensitivity and 98.4% specificity. In high-risk Rh-incompatible cohorts, Jadhav DJP et al.14 demonstrated that neonates requiring phototherapy had significantly higher mean cord bilirubin levels (2.13 ± 0.51 mg/dL vs.^{0}.80 ± 0.58 mg/dL; $p < 0.00001$), with cord bilirubin >1.9 mg/dL showing a strong association with phototherapy requirement.

Cord Bilirubin and Severe Outcomes

In the present study, cord bilirubin levels were higher among neonates who required exchange transfusion (3.73 ± 0.40 mg/dL) and those who developed complications compared to neonates with healthy outcomes (3.14 ± 0.83 mg/dL). However, these differences did not reach statistical significance, likely due to the small number of severe cases (n = 3), limiting statistical power. Krishnan E et al.15 reported that neonates requiring exchange transfusion had cord bilirubin values ranging from 3.17 to 4.05 mg/dL, accompanied by lower cord hemoglobin and higher reticulocyte counts. Gupta N et al.78 emphasized that cord bilirubin was more useful for ruling out severe disease given its very high negative predictive value (99.15%). In contrast, Jadhav DJP et al.14 showed that exchange transfusion was required in 17% of neonates with cord bilirubin >1.9 mg/dL versus only 1.8% among those with lower levels ($p = 0.000328$), indicating that in explicitly high-risk hemolytic cohorts, cord bilirubin becomes highly informative.

Predictive Performance of Cord Bilirubin

In the present study, cord blood bilirubin demonstrated good predictive accuracy for phototherapy requirement, with an AUC of 0.794 (95% CI: 0.711–0.876), and an optimal cutoff of 3.27 mg/dL yielding sensitivity 90.5% and specificity 66.9%. Similar predictive performance has been reported by Kardum D et al.74 (AUC 0.80, cutoff >34 µmol/L), Pradhan A et al.17 (cutoff ≥2.5 mg/dL, sensitivity 84.1%, specificity 88.5%), and Anjanappa S et al.83 (cutoff >2.6 mg/dL, AUC 0.767). Exceptional diagnostic accuracy was reported by Hanasi S et al.77 (cutoff >2.5 mg/dL, sensitivity 100%, specificity 99.3%, AUC ~1.0) and Gupta N et al.78 (AUC 0.948). The comparatively higher cutoff in the present study (3.27 mg/dL) compared to most prior studies is consistent with the higher baseline mean cord bilirubin in our cohort, attributable to the high proportion of ABO/Rh incompatibility. Overall, across diverse populations and study designs, cord blood bilirubin consistently demonstrates good to excellent predictive performance as a screening tool.

Table 29. Overall Comparison of Predictive Utility of Cord Blood Bilirubin Across Studies

Study	Mean Cord Bilirubin (mg/dL)	Cutoff (mg/dL)	Predictive Performance	Key Inference
Our Study	3.16 ± 0.82	3.27	Sensitivity 90.5%, Specificity 66.9%, AUC 0.794	Cord bilirubin is a good early predictor in high-risk neonates
Kardum D et al.74	Higher in hyperbilirubinemia group	>34 µmol/L (~2.0)	Sensitivity 76.9%, AUC 0.80	Independently predicts early hyperbilirubinemia
Pradhan A et al.17	2.47 ± 0.36	≥2.5	Sensitivity 84.1%, Specificity 88.5%	Differentiates pathological from physiological jaundice
Krishnan E et al.15	Pathological: 3.15	>1.8	Sensitivity 72%, Specificity 80%	Rising cord bilirubin parallels increasing jaundice severity
Hanasi S et al.77	2.02 ± 0.36	>2.5	Sensitivity 100%, Specificity 99.3%	Excellent screening marker
Gupta N et al.78	Higher in jaundice group	>2.33	Sensitivity 94.1%, AUC 0.948	Superior to cord albumin for prediction
Sonawane TL et al.80	2.60 ± 0.70	≥3.0	Sensitivity 100%, Specificity 98.4%	Very high NPV makes it ideal for screening
Karthikeyan P16	Pathological: 2.6	2.25	Sensitivity 84%, Specificity 71.1%	Reliably distinguishes pathological jaundice
Jadhav DJP et al.14	Higher with intervention	>1.9	PPV 95.7%, Specificity 97.8%	Strongly predicts severity in hemolytic disease
Anjanappa S et al.83	Phototherapy group: 2.6	>2.6	Sensitivity 63.5%, Specificity 90.5%, AUC 0.767	Predicts intervention even in low-risk neonates

AUC = Area Under Curve; NPV = Negative Predictive Value; PPV = Positive Predictive Value

CONCLUSION

This study demonstrates that cord blood bilirubin is a useful early indicator for predicting the development of significant hyperbilirubinemia in ABO and Rh incompatible newborns. Higher cord bilirubin levels were clearly associated with visible jaundice and an increased need for phototherapy, and ROC analysis confirmed good discriminatory ability with a clinically practical cutoff value of 3.27 mg/dL. Although cord bilirubin alone was not a reliable predictor of exchange transfusion or overall complications—largely due to the small number of severe cases—it showed strong potential as a screening tool to identify neonates who warrant closer observation. When applied thoughtfully, cord bilirubin estimation at birth can support safer early discharge planning, reduce unnecessary prolonged hospital stays, and contribute to timely intervention, thereby helping to minimize the progression to severe hyperbilirubinemia and the need for exchange transfusion.

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