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Cord Blood Bilirubin as an Early Indicator of Neonatal Hyperbilirubinemia: A Prospective Observation Study

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ABSTRACT

Introduction: Neonatal hyperbilirubinemia is a common physiological condition seen in the early postnatal period, affecting up to 60% of term and 85% of preterm neonates.While often benign, delayed identification and treatment of elevated bilirubin levels can result in serious complications such as kernicterus.Given the early discharge practices and limited postnatal follow-up in many settings, a reliable,early predictor of significant jaundice is crucial.Cord blood bilirubin(CBB) estimation offers a simple,non-invasive method for early risk stratification.

Objective: To assess the Cord Blood Bilirubin as an Early Indicator of Neonatal Hyperbilirubinemia

Materials and Methods: This prospective observational study was conducted over 12 months at Oxford Medical College on 65 healthy term neonates (≥37 weeks). Neonates with risk factors such as Rh/ABO incompatibility,cephalohematoma, sepsis,or major congenital anomalies were excluded.Cord blood samples were collected at birth for bilirubin, hemoglobin, and hematocrit levels.Venous samples were obtained on day 2 and day 5 for follow-up bilirubin levels.Significant hyperbilirubinemia was defined as serum bilirubin ≥15 mg/dL at or after 48 hours of life.

Results: Out of 65 neonates, 3 (4.6%) developed significant hyperbilirubinemia. All had CBB ≥3 mg/dL. The sensitivity and negative predictive value of CBB ≥3 mg/dL were both 100%, specificity was 96.8%, and positive predictive value was 60%. No neonates with CBB <3 mg/dL developed hyperbilirubinemia.

Conclusion: Cord blood bilirubin ≥3 mg/dL is a highly sensitive and specific early predictor of significant neonatal hyperbilirubinemia.It is especially useful for ruling out the condition and guiding safe discharge in resource-limited settings.

Keywords*: Cord blood bilirubin,neonatal hyperbilirubinemia,jaundice,bilirubin prediction,newborn screening,kernicterus prevention,early discharge,non-invasive monitoring,term neonates, risk stratification.*

INTRODUCTION

Neonatal hyperbilirubinemia (NH) is a frequently encountered condition in the immediate postnatal period, affecting approximately 60% of term and up to 85% of preterm neonates. Clinically evident as jaundice, this condition is usually benign and self-limiting.[1] However, in a small subset of infants, bilirubin levels can rise to pathologic levels requiring medical intervention. Failure to recognize and manage excessive hyperbilirubinemia in time can result in serious complications such as acute bilirubin encephalopathy and kernicterus—conditions associated with significant neurological sequelae and long-term morbidity.[2]In most healthcare settings, newborns are typically discharged within 48–72 hours of birth, making it challenging to monitor the progression of physiological jaundice, which often peaks between the third and fifth day of life.[3] While the American Academy of Pediatrics (AAP) recommends a follow-up visit within 48–72 hours

of early discharge, adherence to this guideline is not always feasible in resource-constrained settings.[4] Consequently, a considerable proportion of neonates are readmitted with significant jaundice, some of whom may already exhibit early signs of bilirubin-induced neurologic dysfunction (BIND).[5]Various risk factors for developing significant NH have been identified, including prematurity, male sex, polycythemia, ABO and Rh incompatibility, cephalohematoma, exclusive breastfeeding, maternal diabetes, and a family history of neonatal jaundice. Despite these known associations, it remains difficult to predict which infants will develop clinically significant hyperbilirubinemia (defined as total serum bilirubin ≥15 mg/dL).[6] Clinical examination alone, including assessment by the Kramer index, lacks sensitivity and is prone to interobserver variability, especially in infants with darker skin tones.Several approaches have been explored for early identification of at-risk neonates, such as hour-specific bilirubin nomograms, transcutaneous bilirubinometry, and serial serum bilirubin estimations.[7] One such method, cord blood bilirubin (CBB) measurement, has emerged as a potentially valuable, non-invasive, and cost-effective screening tool. Cord blood sampling is routinely performed at birth and offers an opportunity for early bilirubin estimation before clinical signs develop.[8]

Recent studies have evaluated the correlation between CBB levels and the subsequent development of hyperbilirubinemia, with promising results.[8,9] However, variability in predictive thresholds, population characteristics, and clinical practices necessitates further validation in diverse settings.[8,9] The present study was undertaken to evaluate the predictive ability of cord blood bilirubin levels for the development of significant neonatal hyperbilirubinemia in healthy term neonates. By identifying a reliable cutoff level, this study aims to facilitate early risk stratification and timely intervention, potentially reducing the incidence of severe jaundice and associated complications in newborns.

MATERIALS AND METHODS

This hospital-based prospective observational study was conducted in the Department of Pediatrics at Oxford Medical College, Hospital and Research Centre over a duration of 12 months. A total of 65 healthy term neonates delivered within the institution were enrolled after obtaining written informed consent from parents or legal guardians. The study protocol was reviewed and approved by the Institutional Ethics Committee.

Inclusion Criteria

Neonates with a gestational age between 37 and 42 weeks, born as singletons, irrespective of the mode of delivery, and delivered at Oxford Medical College were included.

Exclusion Criteria

Neonates were excluded if they developed jaundice within the first 24 hours of life or had risk factors for severe hyperbilirubinemia such as ABO or Rh incompatibility, cephalohematoma, significant bruising, major congenital anomalies, need for NICU admission, perinatal asphyxia, or clinical evidence of sepsis.

Data Collection and Sampling

Detailed antenatal and perinatal histories were recorded, and each newborn underwent a thorough clinical examination immediately after birth. Gestational age was assessed using the Modified Ballard Score.Umbilical cord blood samples were collected at birth under aseptic precautions and used for the following laboratory investigations: hemoglobin and hematocrit, blood group and Rh typing, and total, direct, and indirect serum bilirubin levels. A cord blood total bilirubin value of ≥3 mg/dL was considered elevated.Venous blood samples were collected at 48 hours and again on the fifth day of life for the assessment of hemoglobin, hematocrit, and serum bilirubin (total, direct, and indirect). Additional bilirubin assessments were performed if clinically indicated.

Sample Handling and Bilirubin Estimation

All collected blood samples were protected from light and stored at 2–8°C until analysis. Serum bilirubin was measured within 12 hours of sample collection using the diazotized sulfanilic acid method. In this method, bilirubin reacts with diazotized sulfanilic acid under acidic conditions to form a pink-coloured azobilirubin complex. The intensity of the colour is directly proportional to the bilirubin concentration. Direct bilirubin, being water-soluble, reacts directly, while indirect bilirubin is solubilized with a surfactant before reaction.Significant neonatal hyperbilirubinemia was defined as a total serum bilirubin level ≥15 mg/dL measured at or after 48 hours of life, which may necessitate phototherapy or further management.

Statistical Analysis

Data were entered in Microsoft Excel and analyzed using SPSS version 26.0. Descriptive statistics such as mean, standard deviation, and percentages were calculated. was assessed using appropriate statistical tests, including chi-square test, independent t-test,. A p-value <0.05 was considered statistically significant.

RESULTS:

In the present study involving 65 neonates (Table 1), three infants (4.6%) developed significant hyperbilirubinemia, defined as a serum bilirubin level of ≥15 mg/dL at 48 hours of life.Maternal characteristics are shown in Table 2. The majority of mothers were aged 21–30 years (73.8%), followed by 18–20 years (15.4%) and 31–40 years (10.8%). The highest incidence

of neonatal jaundice was observed among mothers aged 18–20 years (5.1%). 40 (61.53%) were born to primigravida mothers and 25 (38.46%) to multigravida mothers. The incidence of significant hyperbilirubinemia was slightly higher among neonates born to primigravida mothers (3.2%) compared to those born to multigravida mothers (2.8%).

Among maternal blood groups, A+ (5.6%) and O+ (4.5%) were associated with higher rates of neonatal hyperbilirubinemia, whereas B+ and AB+ groups did not show any such association.

Neonatal characteristics are detailed in Table 3. The gender distribution was nearly equal, with 50.8% males and 49.2% females. Jaundice was slightly more prevalent in females (4.2%) compared to males (3.1%). Most neonates (76.9%) were born between 37–38 weeks of gestation, and all cases of significant hyperbilirubinemia occurred in this group. No cases were reported in neonates born at 39–40 weeks. Regarding birth weight, the majority of neonates weighed between 2.5–3.0 kg (73.8%) and accounted for all jaundice cases. None of the neonates weighing above 3.0 kg developed significant hyperbilirubinemia. Blood group-wise distribution revealed a higher incidence of jaundice among A+ (6.2%) and B+ (4.8%) neonates, while no cases were observed in the O+ and AB+ groups.Table 5 presents the history of neonatal jaundice among previous siblings. Only four neonates (6.2%) had a positive family history, suggesting that family history alone may not serve as a strong predictor in this population. Out of the 65 neonates studied, 38 (58.46%) were delivered by caesarean section and 27 (41.54%) by vaginal delivery. Significant hyperbilirubinemia was observed in 2 neonates (5.3%) born via caesarean section and in 1 neonate (3.7%) delivered vaginally. Among 65 mothers, 30 (46.15%) were anemic and 35 (53.85%) were non-anemic. The incidence of significant neonatal jaundice was 3.3% in babies of anemic mothers and 5.7% in those of non-anemic mothers.This difference indicates that no significant relationship was found between maternal anemia and the development of neonatal hyperbilirubinemia in this study population

The biochemical profile of the study population is summarized in Table 6. The mean cord blood bilirubin was 2.10 ± 0.60 mg/dL, which increased to 10.30 ± 2.40 mg/dL on day 2 and 10.60 ± 2.70 mg/dL on day 5. Hemoglobin and PCV levels showed a physiologic trend of decrease from cord values to day 2 and then a mild rise on day 5. These values remained within expected physiological ranges, indicating the general wellbeing of the study population.Comparative trends are shown in Table 7. Total bilirubin levels showed a progressive rise from cord blood to day 5, which is consistent with the typical postnatal bilirubin dynamics. Meanwhile, hemoglobin and PCV demonstrated a pattern of initial decline by day 2 followed by a slight increase by day 5.

Table 8 illustrates the diagnostic predictability of cord blood bilirubin ≥3 mg/dL for hyperbilirubinemia at 48 hours. All three neonates with significant jaundice had cord bilirubin ≥3 mg/dL (true positives), while two neonates with cord bilirubin ≥3 mg/dL did not develop jaundice (false positives). Importantly, no cases of hyperbilirubinemia were missed among those with cord bilirubin <3 mg/dL (no false negatives), and 60 were true negatives.Diagnostic accuracy is summarized in Table 9. Sensitivity and negative predictive value of cord bilirubin ≥3 mg/dL were both 100%, while specificity was 96.8% and positive predictive value was 60.0%. These findings suggest that cord bilirubin ≥3 mg/dL is a highly sensitive and specific marker for predicting significant neonatal hyperbilirubinemia. The high negative predictive value makes it especially useful as a screening tool to safely rule out the risk of jaundice in neonates, thereby aiding in the safe discharge of healthy term infants and optimizing follow-up strategies.

Table 1. Study Population and Significant Jaundice

Total Number	Significant Jaundice Cases	Percentage
65	3	4.6% (3 out of 65)

Table 2. Maternal Details

Mothers Details	Number of Mothers	Total % (Significant Jaundice %)
Mother Age in Years		
18–20	10	15.4% (5.1%)
21–30	48	73.8% (3.5%)
31–40	7	10.8% (0.0%)
Parity		
Primi	40	61.53% (3.2%)
Multi	25	38.46% (2.8%)
Blood Group		
A+	18	27.7% (5.6%)
B+	20	30.8% (0.0%)
O+	22	33.8% (4.5%)
AB+	5	7.7% (0.0%)
Total	65	100.0%

Table 3. Details of Neonates Studied

Details of Neonates	Number of Neonates	Total % (Significant Jaundice %)
Gender		
Male	33	50.8% (3.1%)
Female	32	49.2% (4.2%)
Weeks of Gestation		
37–38	50	76.9% (4.0%)
39–40	15	23.1% (0.0%)
Birth Weight (kg)		
2.50–3.00	48	73.8% (4.2%)
3.01–3.50	14	21.5% (0.0%)
3.51–4.00	3	4.6% (0.0%)
Blood Group		
A+	16	24.6% (6.2%)
B+	21	32.3% (4.8%)
O+	23	35.4% (0.0%)
AB+	5	7.7% (0.0%)
Total	65	100.0%

Table 5. History of Neonatal Jaundice in Previous Siblings

H/O Neonatal Jaundice	Number of Neonates	Total %
Yes	4	6.2%
No	61	93.8%
Total	65	100.0%

Table 6. Bilirubin, Hemoglobin and PCV Profile of the Study Population

Parameter	Minimum	Maximum	Mean	SD
Cord Blood Total Bilirubin	0.80	3.80	2.10	0.60
Day 2 Total Bilirubin	4.20	18.40	10.30	2.40
Day 5 Total Bilirubin	5.80	19.20	10.60	2.70
Hemoglobin (Cord)	12.50	19.50	15.40	1.50
PCV (Cord)	38.00	56.00	45.70	4.10
Day 2 Hemoglobin	12.80	17.20	14.30	1.20
Day 2 PCV	36.00	54.00	42.80	3.40
Day 5 Hemoglobin	13.00	17.80	14.60	1.10
Day 5 PCV	40.00	50.00	44.10	2.70

Table 7. Comparative Evaluations of Cord, Day 2, and Day 5 Total Bilirubin, Hemoglobin, and PCV

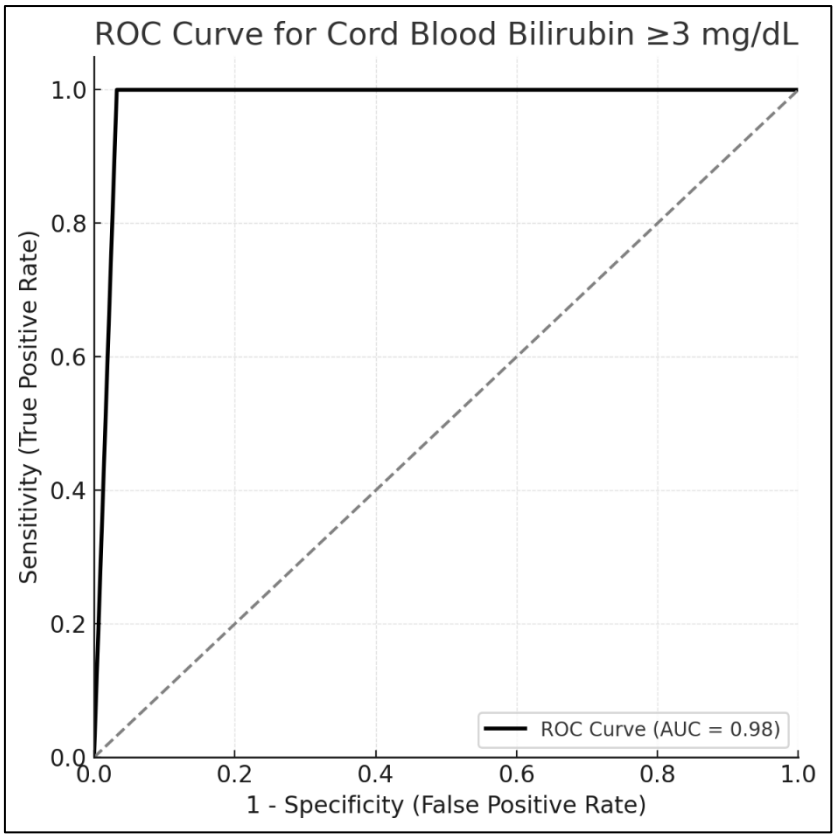
Parameter	Cord	Day 2	Day 5
Total Bilirubin	2.10 ± 0.60	10.30 ± 2.40	10.60 ± 2.70
Hemoglobin	15.40 ± 1.50	14.30 ± 1.20	14.60 ± 1.10
PCV	45.70 ± 4.10	42.80 ± 3.40	44.10 ± 2.70

Table 8: Diagnostic Predictability of Cord Bilirubin ≥3 mg/dL

	Serum Bilirubin ≥15 mg/dL	Serum Bilirubin <15 mg/dL	Total
Cord Bilirubin ≥3 mg/dL	True Positives (TP) = 3	False Positives (FP) = 2	5
Cord Bilirubin <3 mg/dL	False Negatives (FN) = 0	True Negatives (TN) = 60	60
Total	3	62	65

Table 9. Diagnostic Accuracy of Cord Blood Bilirubin >3 mg/dL for Predicting Hyperbilirubinemia at 48 Hours (N = 65)

Parameter	Value
Sensitivity	100%
Specificity	96.8%
Positive Predictive Value	60.0%
Negative Predictive Value	100%



DISCUSSION

Neonatal hyperbilirubinemia remains a common clinical concern, particularly in the first week of life, affecting approximately 60% of term and 80% of preterm neonates. In our study involving 65 full-term healthy neonates, 3 infants (4.6%) developed significant hyperbilirubinemia (total serum bilirubin ≥15 mg/dL at 48 hours), which aligns closely with the 3.5% incidence reported in the earlier study by Ahire et al. (2016) [10] conducted on 113 neonates. Palmer et al. (1983)[11] reported a 10.7% incidence of hyperbilirubinemia in a large cohort of 41,057 newborns, while Phuapradit et al. (1993)[12] documented an incidence of 8.35% in 7,644 neonates. Similarly, Awasthi et al. (1998)[13] reported 12.8% in 274 neonates, and Alpay et al. (2000)[14] found a 12.05% incidence in 498 term neonates. Agarwal et al. (2002)[15] and Knupfer et al. (2005)[16] also observed comparable incidences of 10.3% and 10.6%, respectively. Amar et al. (2005)[17] and Randev et al. (2010)[18] reported incidence rates of 9.5% and 12% respectively in cohorts of 200 neonates.Compared to these findings, the 4.6% incidence observed in our study is markedly lower. One possible explanation is the strict inclusion criteria employed, which focused on healthy term neonates without any hemolytic disease (e.g., Rh or ABO incompatibility). In addition, early identification of risk factors, avoidance of delayed feeding, and the use of cord bilirubin estimation for risk stratification might have contributed to early intervention and reduced progression to significant hyperbilirubinemia.Furthermore, differences in study design, bilirubin threshold definitions, genetic predispositions, feeding practices (especially breastfeeding), and institutional protocols for early discharge and follow-up could all contribute to the variability in reported incidences.The mean cord blood bilirubin in our study was 2.10 ± 0.60 mg/dL, and all three cases of hyperbilirubinemia occurred in neonates with cord bilirubin ≥3 mg/dL. None of the 60 neonates with cord bilirubin <3 mg/dL developed significant jaundice, giving a sensitivity of 100%, specificity of 96.8%, positive predictive value (PPV) of 60%, and negative predictive value (NPV) of 100%. This indicates that cord bilirubin is a reliable early predictor, especially valuable for ruling out hyperbilirubinemia in neonates with low cord levels.When compared to Ahire et al. (2016),[10] who reported a sensitivity of 100%, specificity of 98.17%, PPV of 66.67%, and NPV of 100% using the same cord bilirubin cutoff of ≥3 mg/dL and the same threshold for significant jaundice (≥15 mg/dL), our findings were nearly similar. Knudsen et al. (1989)[19] reported very low sensitivity (13%) but extremely high specificity (99%) with a cutoff of >2.35 mg/dL. In contrast, Zakia Nahar et al. (2009)[20] used a cutoff of ≥2.5 mg/dL and found sensitivity of 77% and specificity of 98.6%, highlighting better balanced diagnostic values. Similarly, Rudy Satrya et al. (2009)[21] reported a sensitivity of 90.5% and specificity of 85% using a threshold of ≥2.54 mg/dL. Our study outperformed all these in terms of sensitivity and NPV, although PPV remained moderate, indicating that while cord bilirubin is excellent at ruling out future hyperbilirubinemia, not all neonates with higher cord bilirubin develop clinically significant jaundice.While prior studies, such as those by Rataj et al. (1994)[22] and Bernaldo et al. (2004),[23] indicated high incidence (85% and 53% respectively) of hyperbilirubinemia in neonates with cord bilirubin >2.5 mg/dL, our study and Ahire et al. reported much lower but clinically significant rates (~3–4%), possibly due to inclusion of non-hemolytic term neonates and early discharge practices in hospitals.The AUC of 0.98 in our ROC analysis further supports the excellent predictive ability of

cord bilirubin. Importantly, the 100% NPV reinforces the potential of using cord bilirubin <3 mg/dL as a safe discharge criterion in resource-limited settings.

CONCLUSION:

Cord blood bilirubin estimation is a simple, non-invasive, and effective tool for early prediction of significant neonatal hyperbilirubinemia. In the present study of 65 healthy term neonates, a cord bilirubin level ≥3 mg/dL demonstrated high diagnostic accuracy, with 100% sensitivity and negative predictive value, and 96.8% specificity. No cases of hyperbilirubinemia were observed among neonates with cord bilirubin <3 mg/dL, underscoring its value as a reliable screening method for safe early discharge in resource-limited settings.The incidence of jaundice was observed to peak between 72 and 96 hours of life, consistent with the physiological bilirubin rise. Jaundice was more frequently seen in neonates born at 37–38 weeks of gestation and those delivered by caesarean section, although the associations were not statistically significant. A slightly higher proportion of primigravida mothers had neonates who developed jaundice compared to multigravida. Importantly, no significant relationship was found between maternal anemia and neonatal hyperbilirubinemia.These findings support the utility of cord bilirubin estimation as an early risk stratification tool and reinforce its relevance in planning follow-up strategies for neonates, particularly in settings where early discharge is common and postnatal monitoring is limited.

Limitation:

The present study was limited by its small sample size and single-center setting, which may restrict the generalizability of findings to broader populations. Hemolytic jaundice cases were excluded, and follow-up was limited to the first five days of life, potentially missing late-onset hyperbilirubinemia. Additionally, confounding factors such as feeding practices and maternal medications were not accounted for.

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