ORIGINAL RESEARCH ARTICLE

Umbilical Cord Blood Bilirubin as a Predictor of Significant Hyperbilirubinemia Requiring Phototherapy among Full-term Healthy Neonates: A Prospective Study

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Abstract

Aim and background: Jaundice is a clinical condition characterized by transient bilirubin conjugation deficiency resulting in neonatal hyperbilirubinemia. Hyperbilirubinemia is defined as a serum total bilirubin concentration greater than 95th centile for the hour of life. Approximately 60% of term and 80% of preterm infants are affected with hyperbilirubinemia, which may lead to complications such as brain neuron damage after several years. Early discharge of healthy-term newborns after delivery has become a common practice, which may be the cause for readmission during the early neonatal period. Universal follow-up within 1–2 days of early discharge, pre-discharge serum total bilirubin, transcutaneous bilirubin measurement, and universal clinical assessment of risk factors of developing jaundice are various strategies to predict significant neonatal hyperbilirubinemia. An association between cord blood bilirubin levels and subsequent risk of neonatal hyperbilirubinemia has been reported. However, the utility of cord blood bilirubin as a screening test to predict subsequent hyperbilirubinemia has been widely debated. We aimed to verify whether cord blood bilirubin at birth could be used as a predictor of significant neonatal hyperbilirubinemia requiring phototherapy among full-term neonates.

Materials and methods: Cord blood bilirubin was estimated in 110 neonates immediately after delivery. These neonates were followed up for the next 3 days and serum total bilirubin was estimated on the third day of life. Neonates with significant hyperbilirubinemia requiring phototherapy were designated as cases and those without hyperbilirubinemia as controls. The association between cord blood bilirubin and serum total bilirubin was determined followed by the identification of the cut-off level of cord blood bilirubin that could predict significant neonatal hyperbilirubinemia requiring phototherapy among term neonates.

Results: Among 100 neonates followed up, 50 developed significant hyperbilirubinemia requiring phototherapy. The mean ± SD of cord blood bilirubin was 2.66 ± 0.65 and serum total bilirubin estimated on day 3 of life was 16.16 ± 1.6 and the difference was statistically highly significant at a *p*-value of <0.00 with paired *t*-test. The cord blood bilirubin and day 3 serum bilirubin were positively correlated with an *r*-value of 0.087. The specificity and sensitivity with cord blood bilirubin of 2.5 mg/dL were 98 and 56%, and with day 3 bilirubin of 11.5 mg/dL was 72 and 100%, respectively.

Conclusion: A cut-off of 2.5 mg/dL in cord blood bilirubin can be used to predict significant neonatal hyperbilirubinemia requiring phototherapy among full-term neonates.

Clinical significance: Umbilical cord blood bilirubin measurement is a simple, economical, and non-invasive method to predict subsequent neonatal hyperbilirubinemia which can aid clinicians in early discharge of normal neonates and selective follow-up of high-risk infants.

Keywords: Cord blood bilirubin, Full-term neonates, Neonatal hyperbilirubinemia, Phototherapy, Serum total bilirubin.

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Introduction

Jaundice is a major cause of concern during the neonatal period manifesting as a result of transient deficiency of conjugation capacity of neonate's liver.[1] Serum total bilirubin that remains in the physiological range reflects a balance between its production and excretion in neonates. However, in some neonates, there occurs an imbalance in these two processes resulting in hyperbilirubinemia.[2] Hyperbilirubinemia is defined as a serum total bilirubin concentration greater than 95th centile for the hour of life. It has been suggested that around 60% of term and 80% of preterm babies suffer from hyperbilirubinemia. This hyperbilirubinemia has been implicated in neuronal damage resulting in severe neurological problems affecting quality of life. In our Indian healthcare setting, there is a trend of early discharge of healthy full-term babies taking into consideration economic factors and the limited availability of healthcare resources. Many times, these neonates get readmitted to the neonatal intensive care unit for hyperbilirubinemia requiring phototherapy. These readmissions pose quite a good number of negative impacts, incurring extra expenses not only to families but also to institutions. Such readmission exposes healthy babies to the

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hospital environment making them prone to acquired infections. Further, these readmissions involve emotional disturbances among mothers, thereby affecting regular breastfeeding practices. There have been constant efforts by pediatricians to identify newborns who are likely to develop neonatal jaundice. Various tools are being tried to assess the risk of significant hyperbilirubinemia such as measurement of serum total bilirubin before discharging the baby, clinical assessment of risk factors, and measurement of transcutaneous bilirubin.[3,4] Babies discharged within 48 hours post-delivery should have a follow-up consultation with a Pediatrician within 2–3 days recommends the American Academy of Pediatrics. This recommendation is slightly difficult to follow in our healthcare setting due to the scarcity of follow-up facilities, thereby imposing a challenge on pediatricians in identifying neonates at risk of hyperbilirubinemia. Various studies have reported an association between umbilical cord blood bilirubin levels and subsequent risk of hyperbilirubinemia.[5] These facts motivated us to undertake this study to determine whether the bilirubin level in the umbilical cord blood of newborns at birth could be used as a predictor of neonatal hyperbilirubinemia requiring phototherapy among full-term babies.

Materials and Methods

A prospective study was conducted in a tertiary hospital attached to a private medical college in central Karnataka to determine the predictive value of umbilical cord blood bilirubin for identifying neonates with hyperbilirubinemia requiring phototherapy. Healthy full-term newborns, 37–42 weeks of gestation as determined by New Ballard Score, both genders delivered by vaginal delivery or cesarean section with birth weight ≥2500 gm were included as study subjects. Infants with major congenital anomalies, sepsis, perinatal hypoxia, low birth weight babies, or gestational age <37 weeks were excluded. A total of 2 mL of cord blood samples were collected from all newborns from the placental side of the cord during delivery in a red color-coded vacutainer. It was ensured that samples were not exposed to light during transportation to the laboratory and during processing and storage. Hemolyzed samples were excluded and bilirubin was estimated by the Daizo method.[6] Measurement of serum total bilirubin was repeated on day 3 with serum samples obtained by the venipuncture procedure. A total of 110 neonates were included; 55 neonates with hyperbilirubinemia requiring phototherapy as cases and 55 neonates without hyperbilirubinemia as controls. The minimum sample size was 52 in each group, calculation was based on the specificity.

Statistical Analysis

The SPSS version 20 was used to perform the statistical analysis. Mean and standard deviation were calculated for quantitative variables, frequency, and percentage for qualitative variables. A paired *t*-test was applied to test the mean difference between cord blood bilirubin and day 3 serum total bilirubin among neonates with and without phototherapy. Pearson's correlation was calculated to test the correlation between umbilical cord blood bilirubin at birth and serum total bilirubin on day 3. Sensitivity and specificity were calculated for umbilical cord blood bilirubin and day 3 serum total bilirubin. The level of significance was set at 5%.

Table 1: Baseline characteristics of the study population

Characteristic	*Frequency (percentage)*
Gender: Males	56 (56%)
Gender: Females	44 (44%)
Neonates without hyperbilirubinemia	50 (100%)
Neonates with hyperbilirubinemia requiring phototherapy	40 (80%)
ABO incompatibility	3 (6%)
Rh incompatibility	7 (14%)
Average birth weight	2.39 kg
Average APGAR score	7.84

Results

The baseline characteristic features of our study subjects (Table 1) were as follows; out of 110 neonates enrolled, 10 did not comply with follow-up criteria and were excluded from the study thus dropout rate was 9.09%. Umbilical cord blood bilirubin was estimated in 100 neonates who were followed up for the first 3 days of life with clinical assessment for jaundice and laboratory estimation of serum total bilirubin on day 3. Among the enrolled neonates, 56 (56%) were boy babies and 44 (44%) were girl babies. All of them were term neonates with an average birth weight of 2.39 kgs and an APGAR score of 7.84. Out of 100 neonates enrolled, 40 (80%) had exaggerated physiological hyperbilirubinemia requiring phototherapy, 3 (6%) neonates had ABO incompatibility with non-O blood group babies delivered by O blood group mothers where naturally occurring anti-A and anti-B antibodies of IgG subtype in mother's serum cross the placenta and can hemolyze fetal erythrocytes, 7 (14%) neonates had Rh incompatibility who were Rh+ve babies born to Rh-ve mothers where maternal Rh antibodies cross placenta and destroy fetal erythrocytes.

Among 100 neonates followed-up, 50 developed significant hyperbilirubinemia requiring phototherapy and these were designated as cases of our study. The mean ± SD of cord blood bilirubin was 2.66 ± 0.65 and serum total bilirubin estimated on day 3 of life was 16.16 ± 1.6 and this difference between cord blood bilirubin and day 3 bilirubin was statistically highly significant at a *p*-value of <0.001 with paired *t*-test. 50 (50%) neonates who did not develop significant hyperbilirubinemia were included as controls. The mean ± SD of cord blood of bilirubin among these neonates was 1.68 ± 0.51 and serum total bilirubin estimated on day 3 of life was 9.92 ± 2.0 and the difference was statistically highly significant at a *p*-value of <0.001 with paired *t*-test (Table 2).

Among the neonates who developed significant hyperbilirubinemia requiring phototherapy, cord blood bilirubin, and day 3 bilirubin were positively correlated with an *r*-value of 0.087 (Table 3) and were statistically significant with Pearson's correlation analysis.

Different cut-off levels of cord blood bilirubin and day 3 bilirubin in predicting hyperbilirubinemia (Table 4) were identified using sensitivity and specificity. With cord blood bilirubin of 2.5 mg/dL, optimal specificity of 98% and sensitivity of 56% was identified. Day 3 bilirubin of 11.5 mg/dL had absolute sensitivity of 100% and specificity of 72%.

Discussion

Neonatal hyperbilirubinemia requiring phototherapy is one of the major factors responsible for the readmission of newborns to

Table 2: Comparison of umbilical cord bilirubin and day 3 bilirubin levels among neonates requiring phototherapy

Phototherapy	*Total bilirubin*	*Mean*	*Std. dev*	*Mean difference*	*p-value**
Yes	UCB	2.66	0.658	−13.500	0.001
	Day 3 TB	16.16	1.683		
No	UCB	1.68	0.513	−8.240	0.001
	Day 3 TB	9.92	2.059		

Unpaired *t*-test

Table 3: Correlation between umbilical cord blood bilirubin and day 3 serum total bilirubin

	Phototherapy	
Pearson's correlation between UCB and day 3 TB	*Yes*	*No*
r-value	0.087	−0.025
p-value	0.548	0.865

Table 4: Sensitivity and specificity of umbilical cord blood bilirubin and day 3 bilirubin in predicting hyperbilirubinemia

Test result variable(s)	*Positive if greater than or equal to*	*Sensitivity*	*Specificity*
UCB	0	100	0
	1.5	100	34
	2.5	**56**	**98**
	3.5	10	100
	5	0	100
Day 3 total bilirubin	1	100	0
	5.5	100	4
	9.5	100	40
	10.5	100	70
	11.5	**100**	**72**
	12.5	96	98
	13.5	88	100
	14.5	86	100
	15.5	78	100
	16.5	42	100
	17.5	22	100
	18.5	4	100
	20	0	100

Umbilical cord bilirubin with a cutoff value of 2.5 mg/dL has 98% specificity and 56% sensitivity and day 3 bilirubin with a cutoff value of 11.5 mg/dL has 72% specificity and 100% sensitivity in predicting neonatal hyperbilirubinemia

the hospital. There is a need to screen all neonates and identify at-risk babies so that healthy babies can be safely discharged at the earliest and at-risk babies can be followed up with optimal utilization of our limited healthcare resources. In this study, we investigated the association between umbilical cord blood bilirubin and the development of subsequent hyperbilirubinemia requiring phototherapy. We observed a preponderance of male babies 56 (56%) toward hyperbilirubinemia when compared with female babies 44 (44%). All of these babies had an average birth weight of 2.39 kg and an APGAR score of 7.84. Among 50 (50%) neonates who developed significant hyperbilirubinemia requiring phototherapy, 3 (6%) neonates had ABO incompatibility, and all three of them were B+ve babies delivered by O+ve mothers. A total of 7 (14%) neonates had Rh incompatibility who were Rh+ve babies born to Rh-ve mothers. Our results are in agreement with that of a study conducted by Zeitoun et al. who reported an association between cord blood bilirubin and hyperbilirubinemia among newborns with ABO incompatibility.[7]

Bilirubin circulating in fetal life is mainly unconjugated because of the restricted capacity of the immature fetal liver to conjugate bilirubin. This unconjugated bilirubin is tightly bound to albumin, which is the predominant bilirubin-binding plasma protein. There occurs no deposition of bilirubin in fetal tissues under normal circumstances. This is because of the immediate transfer of unconjugated bilirubin from fetal to maternal circulation through the placenta. Further, bilirubin produced by the fetus gets excreted by the mother who has a large reserve capacity for bilirubin excretion.[8] Irrespective of blood group incompatibility either with ABO or non-ABO groups between mother and fetus, increased umbilical cord blood bilirubin is an indicator of *in utero* hemolysis.[9] These babies are more prone to develop hyperbilirubinemia requiring phototherapy. Concordance was observed with our results where 40 (40%) neonates did not exhibit any incompatibility with blood grouping and typing (Table 1).

In our study, we observed a positive association between umbilical cord blood bilirubin and serum total bilirubin estimated on day 3 (Table 3) for predicting significant hyperbilirubinemia among term neonates requiring phototherapy. Further sensitivity and specificity analysis was carried out to identify the different cut-off levels of cord blood bilirubin that could be used to predict hyperbilirubinemia (Table 4). Among the series of cut-off values identified for umbilical cord blood bilirubin, the critical value of 2.5 mg/dL had 98% specificity and 56% sensitivity in predicting significant neonatal hyperbilirubinemia. Near absolute specificity of cord blood bilirubin justifies that neonates without having hyperbilirubinemia at the time of delivery could be predicted for the development of subsequent neonatal hyperbilirubinemia requiring phototherapy. The sensitivity of a test is used to identify individuals when they are suffering from disease. Our study subjects, neonates at the time of birth were not suffering from jaundice and this justifies the suboptimal sensitivity of 56% observed in our study. Similarly, Bernaldo and Segre. found that raised unconjugated bilirubin levels in cord blood predicted the severity of jaundice among full-term neonates without any complications when followed up till the third day of life.[10] They observed umbilical cord bilirubin >2 mg/dL indicated a 53% probability for the need for phototherapy. Taksande et al. and Gupta et al. also suggested that a critical level of cord blood bilirubin >2 mg/dL predicted 90% of newborns who developed jaundice.[11,12]

However, Vasudevan et al. observed cord blood bilirubin of 1.5 mg/dL to be strongly associated with neonatal hyperbilirubinemia when compared with other cut-off levels.[13] This is in contrast to our results where specificity was low for a cut-off of 1.5 mg/dL whereas there was absolute sensitivity. Rostami and Mehrabi found that umbilical cord blood bilirubin when raised above 3 mg/dL was not a useful predictor of neonatal jaundice. Similar concordance was observed in our study too (Table 4).[14]

The cut-off level of umbilical cord blood bilirubin varies among different studies. As there is no standard cutoff available for umbilical cord blood bilirubin, we were interested in identifying the critical value of cord blood bilirubin and serum total bilirubin estimated on

Table 5: The receiver operating curve analysis for umbilical cord blood bilirubin and day 3 serum total bilirubin

Test result variable(s)	Area under the curve				
				Asymptotic 95% CI	
	Area	Std. error	p-value	Lower bound	Upper bound
UCB	0.846	0.037	0.000	0.772	0.919
Day 3 serum total bilirubin	0.993	0.005	0.000	0.983	1.000

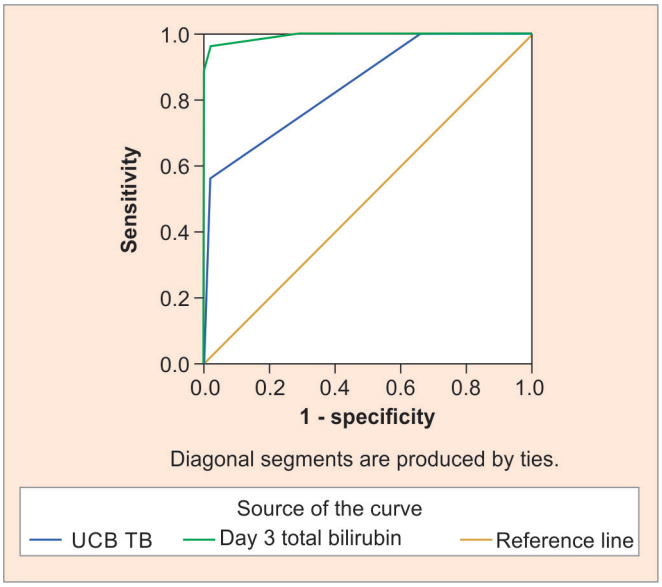

Fig. 1: Receiver operating curve (ROC) for umbilical cord blood bilirubin and day 3 serum total bilirubin

the third day of life that could predict neonatal hyperbilirubinemia requiring phototherapy for our local population. The receiver operating curve (ROC) analysis revealed an area under a curve of 0.846 for cord blood bilirubin with a critical cut-off of 2.5 mg/dL which very well discriminated neonates with hyperbilirubinemia requiring phototherapy from neonates without hyperbilirubinemia (Table 5). Day 3 serum total bilirubin had an area under a curve of 0.99 with a critical cutoff of 11.5 mg/dL discriminating neonates with and without hyperbilirubinemia. As data were not evaluated on nomograms we had to use ROC as this would better reflect the geographic and demographic characteristic features representative of our local population. Our results are in agreement with that of study conducted by Reddy and Umesh and Rehna and Shiyas who reported that cord blood bilirubin of >2.5 mg/dL was a good predictor of neonatal hyperbilirubinemia (Fig. 1).[15,16]

Conclusion

Our results suggest that there occurs a good association between cord blood bilirubin and serum total bilirubin measured on the third day of life and this justifies that cord blood bilirubin could be used as a predictor of neonatal hyperbilirubinemia. A critical cut-off level of 2.5 mg/dL is a useful predictor of significant hyperbilirubinemia requiring phototherapy among full-term neonates.

Clinical Significance

There is a common trend of discharging healthy term babies from the hospital at the earliest and many times these babies get readmitted to the hospital with severe hyperbilirubinemia requiring phototherapy. Cord blood collected during delivery with bilirubin estimated is a useful screening tool for hyperbilirubinemia so that there is scope for discharge of healthy neonates and follow-up of at-risk neonates.

Limitations

Confounding variables of neonatal hyperbilirubinemia such as preterm delivery, prematurity, oxytocin administration, and bruising/cephalhematoma were not studied.

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Ethical Approval

Institutional Ethical Committee approval was obtained (ref no JJMMC/IEC-31-2019).

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