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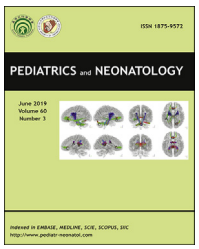

Original Article

Early predictors of neonatal hyperbilirubinemia in full term newborn

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Key Words
cord bilirubin/albumin ratio;
cord serum albumin;
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Background: Reliable predictive markers enabling physicians to identify which newborns will develop significant hyperbilirubinemia have become mandatory for prevention of severe hyperbilirunemia. We aimed at determining the critical cord serum bilirubin and albumin levels and bilirubin/albumin ratio early as reliable markers.

Study design: This prospective study included 175 full-term neonates. Measurement of cord bilirubin, albumin and bilirubin/albumin ratio was done to predict significant hyperbilirubinemia in healthy term newborns based on serum bilirubin measurements made within 5 days of life.

Results: Most cases that developed significant neonatal hyperbilirubinemia (67.9%) had cord albumin level $\leq$ 2.8 gm/dl. Cord Bilirubin/albumin ratio cut off value $>$ 0.61 had a good predictive value with a sensitivity of 100% and specificity of 88.4%, and cord serum albumin cut off value $\leq$ 3.0 mg/dl also had a good predictive value with a sensitivity of 85.7% and specificity of 67.3%.

ROC curve analysis of cord total bilirubin demonstrated that a cut off value of $\geq$1.84 mg/dl had a good predictive value with a sensitivity of 100.0% and specificity of 87.1%.

Conclusion: Cord bilirubin/albumin ratio, serum bilirubin and albumin could be early predictors for neonatal hyperbilirubinemia.

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1. Introduction

Severe hyperbilirubinemia can occur without apparent reason in healthy infants, and some may develop kernicterus.[1] The prevention of poor outcomes necessitates early detection of neonates who are at risk of developing significant hyperbilirubinemia.

Early discharge of healthy term newborns has become a common practice because of advantages including prevention of nosocomial infections, encouragement of early maternal-infant bonding and also lower cost. The American Academic of Pediatrics (AAP) recommends that newborns discharged within 48 h should have a follow-up visit after 48—72 h for any significant jaundice or other problems.[2] In developing countries, the value of follow-up visits after early discharge is questionable as many mothers do not return owing to the distance they need to travel.

Reliable predictive markers enabling physicians to identify which of the newborns discharged early are at increased risk for significant hyperbilirubinemia has become mandatory. Several studies have been performed to assess the ability of cord bilirubin and albumin and first-day bilirubin levels to be tools for screening of subsequent neonatal hyperbilirubinemia.[3—6]

The bilirubin/albumin (B/A) ratio is considered a surrogate parameter for free bilirubin (Bf) and an interesting additional parameter in the management of hyperbilirubinemia.[7] It offers the clinician a reasonable measure of bilirubin binding to albumin until unbound bilirubin or albumin binding reserve can be measured clinically with accuracy and precision.[8]

To our knowledge, there are no documented studies evaluating the significance of cord B/A ratio in predicting neonatal hyperbilirubinemia. In the present study we aimed at determining the critical cord serum bilirubin and albumin level and bilirubin/albumin ratio that predict significant hyperbilirubinemia in healthy term newborns based on serum bilirubin measurements made within 5 days of life.

2. Materials and methods

This is a prospective cohort study that included 175 neonates born in Cairo University Hospital, from July 2015 to June 2016. All neonates involved were full term (gestational age ranging from 37 to 41 weeks) of either gender without any significant illness or major congenital malformations. The neonates with conditions that could aggravate hyperbilirubinemia (sepsis, respiratory distress syndrome, asphyxia, diabetic mothers, or intrauterine growth retardation) or cholestatic jaundice were excluded from the study.

Participants were subjected to detailed history-taking including maternal medical diseases, consanguinity, siblings by hyperbilirubinemia, mode of delivery, Apgar score, oxytocin use, and type of feeding. Thorough physical examination of neonates was done with assessment of gestational age by new Ballard score and birth weight by growth curves. The use of phototherapy or exchange transfusion were recorded as indicated by the American Academy of Pediatrics guidelines for management of neonatal hyperbilirubinemia.[2]

In the delivery room, 3—5 ml of cord venous blood were collected after clamping the umbilical cord with 2 clamps, the second placed 4—6 inches away from the first. The collected sample was tested for serum albumin level using bromocresol green method (BCG), serum bilirubin (total and direct) level by Colormetric method, hemoglobin concentration using cell counter T 660, and reticulocytic count done manually after staining with brilliant cresyl blue and examined under oil emersion lens. Blood groups (ABO, Rh) were determined for newborns and mothers.

Follow up was done on days one, three and five of life by assessment of serum bilirubin level for all cases. Significant hyperbilirubinemia was defined as the need of phototherapy or exchange transfusion based on the American Academy of Pediatrics guidelines for management of neonatal hyperbilirubinemia.[2]

2.1. Sample size

Sample size calculation was based on the sensitivity of cord blood albumin and bilirubin in predicting the occurrence of indirect neonatal hyperbilirubinemia. We planned to study the independent cases and controls with 1 control(s) per case. Prior data indicated that the average sensitivity of cord blood albumin in predicting later occurrence of neonatal hyperbilirubinemia was approximately 77%[3] while that of cord blood bilirubin was 85%.[4] If the true sensitivity differed by 10%, we needed to study 21 cases and 21 control subjects to be able to reject the null hypothesis for cord blood albumin and we needed to study 18 cases and 18 control subjects to be able to reject the null hypothesis for cord blood bilirubin with 80% power. Thus, we took cord blood samples from all newborns until we had a minimum of 21 cases with significant indirect neonatal hyperbilirubinemia. The remaining samples without significant indirect neonatal hyperbilirubinemia were considered controls with a minimum of 21 babies. We used uncorrected chi-squared statistics to evaluate this null hypothesis with setting type I error probability to 0.05. Calculations were done using *Flahault* equation.[9]

2.2. Statistical analysis

Data were entered on the computer using Microsoft Office Excel Software program (2010) for Windows, then transferred to the Statistical Package of Social Science Software (SPSS) program, version 23 to be statistically analyzed. Data were summarized using range, mean, standard deviation, median and percentiles for quantitative variables or frequency and percentage for qualitative ones. Comparison between groups was performed using Mann—Whitney test for quantitative variables while comparison for qualitative variables was performed through Chi square or Fisher's exact test. Receiver operating characteristics (ROC) curve analysis was performed to explore the discriminant ability of different cord measures in predicting neonatal jaundice. P values less than 0.05 were considered statistically significant. Graphs were used to illustrate some information.

3. Results

The study population included 175 neonates with a mean gestational age of 37.9 (±0.9) weeks and a mean birth weight of 2.9 (±0.3) kg. Of these, 28 neonates (16%) developed significant hyperbilirubinemia (group 1) and 147 neonates (84%) did not (group 2).

Among the 28 neonates who developed significant neonatal hyperbilirubinemia, 16 were males and 12 were females; 21 cases were delivered by caesarean section and 6 cases received oxytocin for induction of labour. As regards the 147 neonates who did not develop significant neonatal hyperbilirubinemia, 73 were males and 74 were females; 105 cases were delivered by caesarean section and 29 cases received oxytocin for induction of labour.

Group 1 infants had statistically significant higher cord reticulocytic count [(3.4 ± 1.3%) versus (2.1 ± 0.8%)] than those in group 2 ($p < 0.001$) with a diagnosis of Rh incompatibility in 10.7% of the cases (3 patients out of 28) and ABO incompatibility in 14.3% of the cases (4 out of 28 patients) in group 1 versus 1.4% and 4.1% respectively in group 2.

Cases with significant neonatal hyperbilirubinemia (group 1) had statistically significantly higher cord total bilirubin [(2.4 ± 0.2 mg/dl) versus (1.4 ± 0.4 mg/dl)] ($p < 0.001$), significantly lower cord albumin [(2.8 ± 0.3 gm/dl) versus (3.3 ± 0.5 gm/dl)] ($p < 0.001$), and significantly higher cord B/A ratio [(0.86 ± 0.14) versus (0.44 ± 0.19)] with $p < 0.001$ (Table 1).

Table 1 Comparison of laboratory data of cases with significant and insignificant neonatal hyperbilirubinemia.

	Group 1 (n = 28)	Group 2 (n = 147)	P value
Cord hemoglobin (gm/dl)			
Range	15.3 – 18.5	14 – 18.2	0.070
Mean ± SD	16.5 ± 1	16 ± 0.9	
Median	16.3	16.2	
Cord reticulocytes			
Range	1 – 7	1 – 4	**<0.001**[#]
Mean ± SD	3.4 ± 1.3	2.1 ± 0.8	
Median	3	2	
Cord total bilirubin (mg/dl)			
Range	1.9 – 3	0.6 – 2.6	**<0.001**[#]
Mean ± SD	2.4 ± 0.2	1.4 ± 0.4	
Median	2.4	1.3	
Cord direct bilirubin (mg/dl)			
Range	0.2 – 0.7	0.1 – 0.5	**0.029**[#]
Mean ± SD	0.3 ± 0.1	0.3 ± 0.1	
Median	0.3	0.2	
Cord albumin (gm/dl)			
Range	2.4 – 3.6	1.8 – 4.8	**<0.001**[#]
Mean ± SD	2.8 ± 0.3	3.3 ± 0.5	
Median	2.7	3.3	
Cord bilirubin/Albumin ratio			
Range	0.62 – 1.2	0.14 – 1	**<0.001**[#]
Mean ± SD	0.86 ± 0.14	0.44 ± 0.19	
Median	0.88	0.4	

SD = standard deviation, significant P value < 0.05.

Total serum bilirubin levels measured on days 1, 3 and 5 were significantly higher ($P < 0.001$) in group 1 [(8.8 ± 1.5 mg/dl versus 4.1 ± 1.2 mg/dl)], [(15.4 ± 2.5 mg/dl versus 6.7 ± 2.8 mg/dl)] and [(13.8 ± 5.1 mg/dl versus 4.4 ± 2.4 mg/dl)] respectively. However, the hemoglobin concentration showed no significant difference between groups.

The cases with significant hyperbilirubinemia were all managed with phototherapy whether intensive (82.1%) or conventional (17.9%). Intravenous immunoglobulin (IVIG) was indicated in 14.3% of the cases and none required exchange transfusion.

Among the neonates that developed significant hyperbilirubinemia, 67.9% had low cord serum albumin <2.8 mg/dl, 25% had it ranging between 2.8 and 3.3 mg/dl and only 7% had a level over 3.3 mg/dl.

For the prediction of significant neonatal hyperbilirubinemia, a cut off value of cord serum bilirubin of 1.84 mg/dl was chosen on the basis of the ROC curve analysis. The area under the curve was 0.95 indicating high significance. The cord serum bilirubin of 1.84 had a sensitivity of 100%, specificity of 87.1%, positive predictive value of 59.6% and negative predictive value of 100% in the prediction of neonatal hyperbilirubinemia (Fig. 1) (Table 2).

The optimum cut off value for cord serum albumin as shown by the ROC curve for neonates with significant indirect hyperbilirubinemia was 3 gm/dl with a sensitivity of 85.7% and specificity of 67.3%, negative predictive value of 96.1% and positive predictive value of 33.3% (area under the curve of 0.825) (Fig. 2) (Table 2). Also, the cord B/A ratio cut off value of >0.61 had a good predictive value for neonates that developed significant neonatal hyperbilirubinemia with a sensitivity of 100%, specificity of 88.4%, positive predictive value of 62.2% and negative predictive value of 100.0%. The area under the curve is 0.936 indicating high significance (Fig. 3) (Table 2).

4. Discussion

The need for early prediction of jaundice has become increasingly important for identifying those babies at risk of neonatal hyperbilirubinemia considering the severe neurological morbidities caused by bilirubin toxicity.

The current AAP guidelines for managing healthy jaundiced term and near-term newborns recommends the use of the total bilirubin concentration/albumin ratio in addition to the TBC; however, it has not been widely used by clinicians.[2] In the current study the mean cord B/A ratio was significantly higher in neonates who developed neonatal hyperbilirubinemia than in those who did not (0.86 ± 0.14 versus 0.44 ± 0.19). ROC curve analysis of cord B/A ratio demonstrated that a cut off value ≥ 0.61 mg/dl had a good predictive value with a sensitivity of 100.0%, specificity of 88.4%, and positive predictive value of 62.2%.

Ahlfors et al. suggested that, in the absence of an available assay for free bilirubin (Bf), the bilirubin/albumin ratio B/A might provide a better estimate of Bf because it contains 2 of the 3 factors determining Bf (TSB, albumin and the albumin binding affinity).[10] However, the value of B/A ratio may be reduced because of some factors that influence the intrinsic albumin-bilirubin binding constant (it

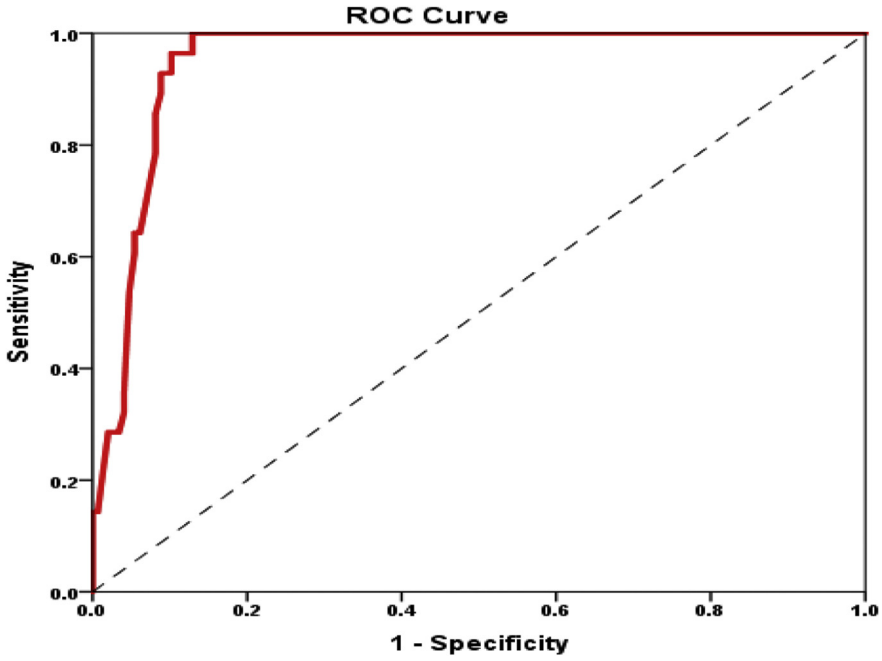

Figure 1 ROC curve analysis to explore the discriminant ability of cord total bilirubin in predicating significant hyperbilirubinemia.

Table 2 Comparison between ROC curves.

	Cord total bilirubin	Cord albumin	Cord B/A Ratio
Sensitivity	100.0%	85.7%	100.0%
Specificity	87.1%	67.3%	88.4%
PPV	59.6%	33.3%	62.2%
NPV	100.0%	96.1%	100.0%
Cut-off	≥1.84	≤3.0	≥0.61
P value	<0.001	<0.001	<0.001

PPV = positive predictive value, NPV = negative predictive value, significant P value < 0.05.

may be decreased by drugs (e.g, ceftriaxone)[7] and the presence of other plasma constituents that bind unconjugated bilirubin (as apolipoproteins and alfa fetoprotein).[11]

The current study demonstrated a highly significant difference between the cord serum bilirubin levels in the 2 studied groups. They were lower in neonates who did not develop significant hyperbilirubinemia (1.4 ± 0.4 mg/dl) versus those who developed it (2.4 ± 0.2 mg/dl). This is consistent with Ipek et al. who found that the mean cord serum bilirubin was also lower in babies who developed neonatal hyperbilirubinemia versus those who did not (1.64 ± 0.41 mg/dl versus 2.05 ± 0.9 mg/dl).[12] Several studies reported lower mean cord serum bilirubin levels in neonates who developed hyperbilirubinemia.[3,4,13]

ROC curve analysis of cord total bilirubin demonstrated that a cut off value ≥ 1.84 mg/dl had a good predictive value with a sensitivity of 100.0%, specificity of 87.1%, and positive predictive value of 59.6%. Rajpurohit et al. reported a cord blood bilirubin cut off value > 2 mg/dl had a sensitivity of 90%, specificity of 53.89%, positive predictive value of 17.8% and negative predictive value of 98% in predicting the risk of neonatal hyperbilirubinemia.[5] Knüpfer et al. reported that a cord bilirubin cut off level of 1.76 mg/dl for predicting hyperbilirubinemia had a sensitivity of 70.3% and a negative predictive value of 65.6% and they concluded that cord blood bilirubin could be used as an early predictor of neonatal jaundice.[14] Dwarampudi and Ramakrishna reported that neonates with cord bilirubin level less than 2 mg/dl were in a safe zone with respect to development of subsequent hyperbilirubinemia.[15]

Albumin helps in hepatic transportation of bilirubin and its clearance. Low serum albumin level decreases bilirubin clearance and thus increases significant hyperbilirubinemia.[16] The ability of cord albumin to act as a tool for predicting neonatal jaundice was assessed in the current study. There was a significantly higher cord serum albumin level in neonates who did not develop neonatal hyperbilirubinemia in comparison to those who did. 67.9% of cases that developed significant neonatal hyperbilirubinemia had cord albumin level ≤2.8 gm/dl. Burtis et al.[17] stated that the lower normal limit for cord serum albumin in term babies was 2.8 gm/dl; and Reshad et al.[18] found that in the term group, 19 (61.2%) newborns with cord serum albumin <2.8 g/dl developed neonatal hyperbilirubinemia. Moreover, similar results were reported by several researchers[3,4,19] that found cases with low cord albumin <2.8 gm/dl developed more significant hyperbilirubinemia requiring phototherapy and exchange transfusion.

Receiver operating characteristics (ROC curve) analysis demonstrated that cord serum albumin cut off value ≤ 3.0 mg/dl had a good predictive value with a sensitivity of 85.7% and specificity of 67.3%. However, Rajpurohit et al. reported a lower cord blood albumin level (≤2.6 gm/dl) to have a sensitivity of 80% and specificity of 86.67% in predicting the risk of neonatal hyperbilirubinemia.[5] Aiyappa and colleagues found the sensitivity of cord albumin to detect hyperbilirubinemia to

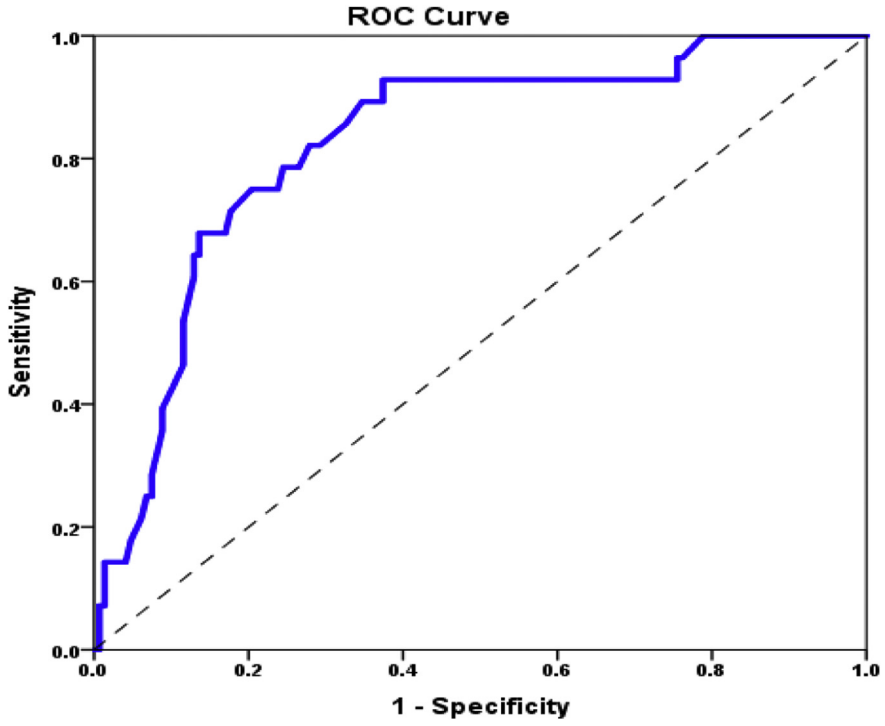

Figure 2 ROC curve analysis to explore the discriminant ability of cord albumin in predicating significant hyperbilirubinemia.

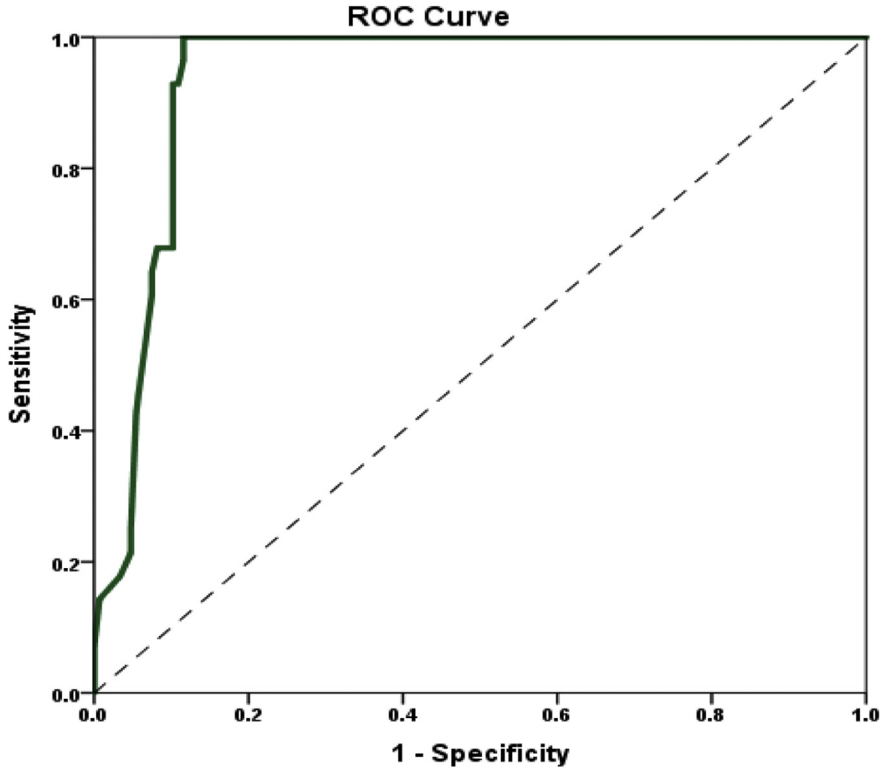

Figure 3 ROC curve analysis to explore the discriminant ability of B/A ratio (cord) in predicating significant hyperbilirubinemia.

be 71.8%, while specificity was 65.1%.[6] Similarly, Pahuja et al. stated a fair predictive value of cord albumin for development of neonatal hyperbilirubinemia of 75%.[20] Also Dwarampudi and Ramakrishna suggested that cord albumin levels (>2.8 gm/dl) were probably safe to discharge a neonate in respect to the risk of development of neonatal hyperbilirubinemia.[15]

With lack of studies done on cord B/A ratio as an early predictor of significant hyperbilirubinemia, this work opens

the window for further studies to be performed in this field and we are aware that larger scale trials including preterm neonates are needed.

In this study cord serum B/A ratio proved to predict the development of significant neonatal hyperbilirubinemia. Infants with either cord serum total bilirubin $\geq$1.84 mg/dl, cord serum albumin $\leq$3.0 gm/dl or cord serum B/A ratio $\geq$0.61, were at risk of developing significant indirect neonatal hyperbilirubinemia needing interventions. These can be considered possible early predictors for neonatal hyperbilirubinemia.

We recommend measurement of cord serum albumin, albumin and bilirubin/albumin ratio in all healthy term babies at delivery to prevent dangerous consequences of hyperbilirubinemia as acute bilirubin encephalopathy.

Conflict of interest

Nil.

Ethical approval

The study was approved by the Ethics Committee of Pediatric Department, Faculty of Medicine, Cairo University.

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