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Predictive Value of Cord Blood Bilirubin for Hyperbilirubinemia in Term Neonates with ABO Incompatibility

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ABSTRACT

Introduction: Jaundice affects 60% of full-term and 80% of preterm neonates. Feto-maternal ABO incompatibilities occur in 20-25% of pregnancies, but severe haemolytic disease develops in only 10%. ABO-incompatible babies have double the risk of jaundice requiring treatment and a 5-10 times increased risk of exchange transfusions.

Objective: Aim of this study was to determine if cord blood bilirubin (CBB) can predict hyperbilirubinemia in term babies with ABO setup prior to discharge that would help to decrease readmission and subsequently complications due to jaundice.

Methods: A hospital-based prospective observational study was conducted at Patan Hospital. Cord blood group and bilirubin levels were collected from newborns of O positive mothers. Babies with A positive and B positive blood groups had their CBB levels measured and were followed daily for 72 hours. Serum bilirubin was tested based on clinical judgment to detect hyperbilirubinemia and treated per NICE guidelines. Data were analysed using SPSS v21.0, Mann-Whitney test, ANOVA, and ROC analysis.

Results: Among 66 cases, significant hyperbilirubinemia was seen in 7 neonates (10.6%). It was more common in B positive babies (12.2%) compared to A positive (8%). Mean CBB was 3.45 ± 0.57 in those developing significant hyperbilirubinemia. A CBB of 2.95 mg/dL had the highest sensitivity (86%), specificity (94%), and negative predictive value (98%) for predicting hyperbilirubinemia in ABO setup babies.

Conclusion: Term babies with ABO incompatibility and CBB <2.95 mg/dL are unlikely to need further evaluation, while those with cord bilirubin level ≥2.95 mg/dL should have frequent early follow-ups.

Keywords: ABO incompatibilities; hyperbilirubinemia; neonatal jaundice; phototherapy.

INTRODUCTION

Jaundice affects 60% of full-term and 80% of preterm neonates.[1,2]ABO incompatibilities occur in 20 – 25 % of pregnancies, with severe haemolytic disease in 10%.[3] ABO incompatible babies have double the jaundice risk and 5-10 times higher exchange transfusion risk.[2] with reduced Rhesus D alloimmunization due to anti-D prophylaxis, ABO incompatibility is now the

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major cause of immune haemolytic disease of new born in developed countries.[4] In Canada, ABO incompatibility is the leading cause of severe hyperbilirubinemia, followed by G6PD deficiency.[5] In India, main causes of neonatal jaundice are exaggerated physiological jaundice, ABO-incompatibility, Rh-incompatibility, Idiopathic and others .[6]

In Nepal, 56% of neonatal jaundice cases are physiological, with ABO incompatibility being the most common pathological causes followed by sepsis.[7] Predicting of significant jaundice at birth offers an option to identify neonates at risk of hyperbilirubinemia. Methods, like transcutaneous bilirubin measurement and cord blood bilirubin (CBB) estimation, have been studied for this purpose.[8]

No published studies on CBB's predictive value for neonatal jaundice in Nepal found by researcher. Thus, study aims to evaluate CBB's predictive value for hyperbilirubinemia in pregnancies involving group O-mothers and A- or B term babies, determining a CBB cutoff for safe discharge.

METHODS

This is a prospective observational study conducted in department of Pediatrics, Patan Academy of Health Sciences (PAHS), Lalitpur from 12th of February 2021 to 11th January 2022. Ethical clearance was obtained from Institutional review committee of PAHS.

Physician attending the cesarean section got the information about mother's blood group from her antenatal checkup file. Consent was taken from the husband of the patient undergoing cesarean section at the time of shifting to operation theatre. Once the umbilical cord was clamped, it was wiped with povidone iodine. And a needle was inserted into the vein in umbilical cord to collect the blood. Cord blood was collected by the pediatrics doctor on duty attending the cesarean section. The blood was collected in biochemistry and Ethylenediaminetetraacetic acid (EDTA) vials. The blood grouping was analyzed immediately (Blood Grouping of baby was done by using known antisera with slide and tube methods) and cord bilirubin of only those babies whose blood group came out A positive and B positive was sent. These tests were a part of the revised protocol of pediatric department.

The newborn enrolled in the study was daily followed till 72hrs in every morning rounds by the researcher conducting the study and Serum bilirubin was sent according to the doctor's decision and clinical judgement to detect hyperbilirubinemia.

The newborns identified with clinical jaundice had their serum bilirubin sent. Serum bilirubin was sent to lab in capillary tube and estimated by standard lab methods on Auto analyzers by Diazo method.

The result of the total serum bilirubin (TSB) value was interpreted and treatment was started

on the basis of the age wise hour specific TSB threshold graphs given by National Institute for Health and Care Excellence (NICE) guideline.[9] The same guideline for phototherapy is included in the hospital protocol of Patan Hospital, Department of Pediatrics. Hour specific TSB threshold graphs are present for different gestational age beginning from 23 weeks till ≥38 weeks of gestation. The treatment threshold graphs applicable in the study are those of 37 and ≥38 weeks. The X axis in the graphs represent time since birth in days and the Y axis represent TSB level in micromol/litre. The Blue curve in the graph represents the threshold for phototherapy and the red curve represents threshold for exchange transfusion. The SI unit for total serum bilirubin is mg/dl and the conversion factor to convert the value into micromol/litre is 17.1. But for simplicity TSB value in mg/dl as given by laboratory is converted into micromol/litre by multiplying the value by 17 as practiced in our hospital.

Babies who on regular follow-up developed jaundice as per judgement of attending physician as well as clinical assessment had their serum bilirubin levels sent and if their serum bilirubin levels required treatment (as per NICE guidelines) they were grouped into babies with significant hyperbilirubinemia. Other necessary information's were taken from neonate assessment sheet.

Presence or absence of sepsis was defined by the treating doctor on the basis of the clinical status, laboratory parameters and culture reports of the babies. Presence of cephalhematoma was recorded following examination of the babies. Babies who were found to develop cephalhematoma or sepsis (as per department protocol) later were excluded from the study.

Convenient sampling was used in this study where all the neonates admitted in 1-year period were enrolled in the study. Data was collected on preformed proforma and entered in Microsoft Excel 2013 and statistically analyzed using SPSS Statistics for Windows, v21.0. Continuous variables were expressed as mean or median and Fischer exact test was applied to identify the associated risk factors for significant hyperbilirubinemia. Independent sample t test was used to see if higher cord bilirubin level is associated risk of developing significant hyperbilirubinemia. Receiver operating characteristic (ROC) curve was generated cord bilirubin level as test variable to predict significant hyperbilirubinemia. Optimum cutoff value was obtained from the ROC curve. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the cutoff score. Mann–Whitney U test was used to compare the scores between the significant and no hyperbilirubinemia groups. Results were expressed with a corresponding 95% confidence interval (CI). A P-value of <0.05 was taken to be significant.

RESULTS

A total of 66 cases were enrolled in the study, which shows demographics table as follow in table 1.

Table 1: Baseline characteristics of neonates.

Parameter	N	%
GESTATIONAL AGE		
37 to 37+6 WOG	22	46%
38+1 to 39 +6 WOG	40	36%
> 40 WOGS	4	18%
BIRTH WEIGHT		
>4kg	1	1.5%
3.5 to 4kg	5	7.5%
2.5-3.5kg	51	13.5%
<2.5kg	9	77.5%
SEX		
Male	37	56%
Female	29	44%
BLOOD GROUP		
A positive	25	38%
B positive	41	62%

Incidence of significant hyperbilirubinemia in this study was 7 neonates (10.6%). The incidence of significant hyperbilirubinemia was higher in female babies but was not statistically significant (p value 0.363) (Table 2). The incidence of significant hyperbilirubinemia was higher in 38 to 39^{+6} but was not statistically significant (p value 0.703) (Table 3). The incidence of significant hyperbilirubinemia was higher in babies with blood group B but was not statistically significant (p value 0.7009) (Table 4). The incidence of significant hyperbilirubinemia was higher in babies with birth weight 2.5 to 3.5kg but was not statistically significant (p value 0.5668) (Table 5).

Table *2:* Association of Sex as risk factor for significant hyperbilirubinemia.

SEX OF BABY	SIGNIFICANT HYPERBILIRUBINEMIA	PERCENTAGE	P VALUE
Male (37)	3	8.1%	0.363
Female (29)	4	13.79%	

Table 3: Association of gestational age as risk factor for significant hyperbilirubinemia.

Gestational Age (WOG)	SIGNIFICANT HYPERBILIRUBINEMIA		TOTAL	p- value
	Yes	No		
37 to 37^{+6}	3 (13.6%)	19 (86%)	22(100%)	0.703
38 to 39^{+6}	4 (10%)	36 (90%)	40(100%)	

>40	0	4(100%)	4(100%)	
TOTAL	7 (10.6%)	59 (89.3%)	66 (100%)	

Table 4: Association of Blood group of babies as risk factor for significant hyperbilirubinemia

BLOOD GROUP	SIGNIFICANT HYPERBILIRUBINEMIA		TOTAL	p- value
	Yes	No		
A POSITIVE	2 (8%)	23 (92%)	25 (100%)	0.7009
B POSITIVE	5 (12.2%)	36 (87.8%)	41(100%)	
TOTAL	7 (10.6%)	59 (89.3%)	66 (100%)	

Table 5: Association of Birth weight as risk factor for significant hyperbilirubinemia

BIRTH WEIGHT	SIGNIFICANT HYPERBILIRUBINEMIA		TOTAL	p- value
	Yes	No		
< 2.5kg	2 (22.2%)	7 (78.8%)	9(100%)	0.5668
2.5 to 3.5 kg	5 (9.8%)	46 (90.2%)	51(100%)	
3.5 to 4 kg	0	5(100%)	5(100%)	
>4kg	0	1(100%)	1(100%)	
TOTAL	7 (10.6%)	59 (89.3%)	66 (100%)	

Table 6: Mean cord blood bilirubin among significant and insignificant hyper bilirubinemia

Significant Hyperbilirubinemia	Number	Percentage	Maximum	minimum	Mean CBB (mg/dl)	SD	P value
YES	7	10.6%	4.1	2.3	3.45	0.57	0.015
NO	59	89.4%	3.9	0.7	2.16	0.65	

Mean cord bilirubin level was 3.45 ± 0.57 in groups developing significant hyperbilirubinemia and 2.16 ± 0.65 in groups not developing significant hyperbilirubinemia (statistically significant p value 0.015) (Table 6).

Receiver operating characteristic (ROC) curve was generated with cord bilirubin level as the test variable to predict significant hyperbilirubinemia (Figure 1). The area under the curve (AUC) for cord bilirubin level was 0.92 (which indicates the prediction accuracy of

92%. ROC curved showed that cord bilirubin level of 2.95 had sensitivity of 86%, Specificity of 94%, Positive predictive value of 67% and Negative predictive value of 98%.

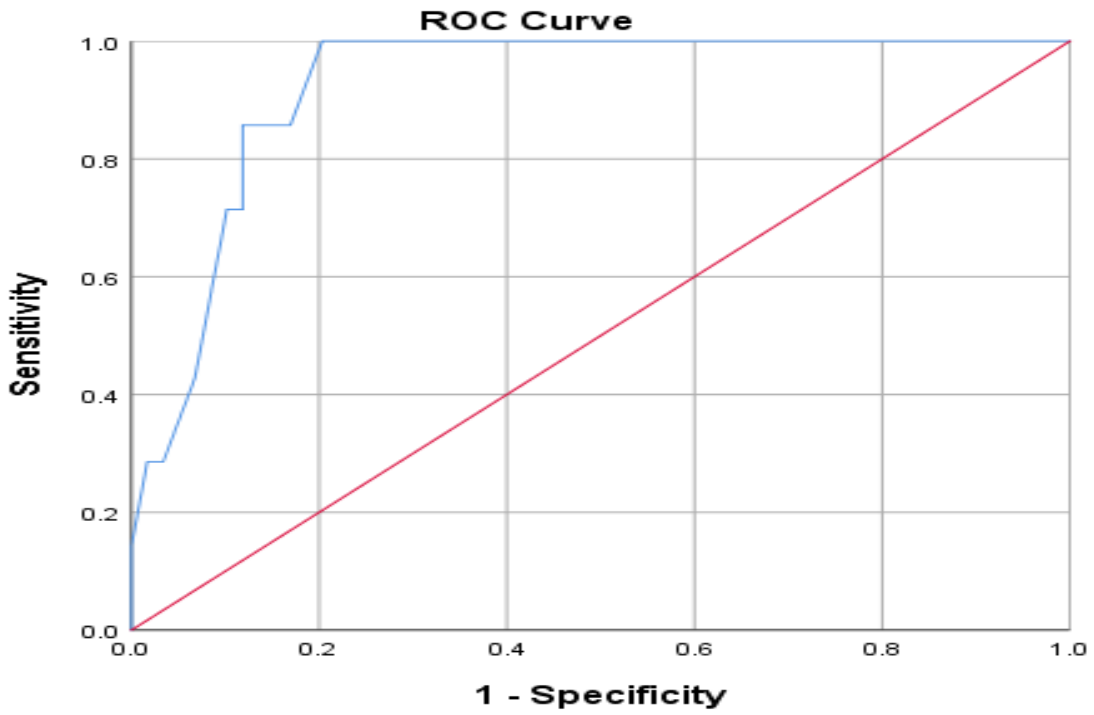

Figure 1: ROC curve to assess the ability of umbilical cord serum total bilirubin to predict significant hyperbilirubinemia

DISCUSSION

Our aim was to find a correlation of CBB level with subsequent Neonatal Hyperbilirubinemia in A and B blood group babies born to O blood group mothers and to find a cut-off level of CBB for predicting the development of Neonatal Hyperbilirubinemia. We chose cord bilirubin because it is a non-invasive way, simple, and the results are available within few hours after birth and estimation of bilirubin can be done even in resource limited rural areas. In our study among 66 term newborns were enrolled, 7 (10.6%) newborns developed significant hyperbilirubinemia. This result is little lower than studies conducted by Bernard AJNB [10] and Segre CADM and Nahar Z et al studies [11] in babies born to O positive mother who reported an incidence of 15% and 15.5% respectively. [12]

In our study the incidence of significant hyperbilirubinemia was higher in blood group B but was not statistically significant (p value 0.467). This is in consistent with the fact that among 66 babies (60% babies had blood group B positive). The literature is inconsistent with

regard to the degree of hemolysis and the incidence and severity of hyperbilirubinemia among O-A and O-B pairs. Kaplan et al study using ETCOc as predictor for hyperbilirubinemia showed O-B blood group had more significant and severity of Jaundice. [12] Our study showed a positive correlation only and observations were not statistically significant.

In our study the incidence of significant hyperbilirubinemia was higher in female babies but was not statistically significant (p value 0.363). Literature review reported variable sex distribution with some stating disease as more common in female babies and some males (43-44). Hodr R, in 2008 observed that among neonates treated successfully by phototherapy, boys prevailed significantly and there was significantly higher prevalence of girls among the most severe forms of ABO hemolytic disease.[13] Dufour DR in a retrospective analysis of 254 cases found that sex, race, gravidity, birth weight and blood type of the infant did not have any significant relationships to outcome. [14]

In our study, out of 7 newborns who developed significant hyperbilirubinemia, 28 % were less than 2.5 kg, 72% were between 2.5-3.50kg and which was not statistically significant (p value 0.17). This observation was comparable with the studies done by Adelia and Canceicao, Dufour D.R et al, in which birth weight was not determining factor for development of neonatal hyperbilirubinemia in term neonates. [14]

In our study, the relationship between the clinical sensitivity and specificity with respect to every possible cut off value of cord blood bilirubin was constructed using ROC curve. The point with maximum sensitivity (86%) and specificity (94%) was found at value of more than 2.95 mg/dl, with area under curve 0.95. Positive predictive value and negative predictive value at this point were found to be 67.06% and 98% respectively. A 98% Negative Predictive Value in the present study suggests that in healthy term babies with ABO, Cord Blood Bilirubin < 2.95 mg/dl can help to identify those newborns who are unlikely to require further evaluation and intervention. Babies with Cord Blood Bilirubin level ≥ 2.95 mg/dl should be followed more frequently to reduce mortality and morbidity due to Neonatal hyperbilirubinemia.

Naharb Z et al in their study, which included both term and preterm newborns, concluded that a critical value of cord blood bilirubin more than 2.5 mg/dl had the high sensitivity (77%) and specificity (98.6%) for predicting neonatal hyperbilirubinemia.[11] In contrary to our study this study included preterm too(24%) .Significantly higher percentage of pre-term babies developed hyperbilirubinemia which may be the reason for low cut off value of cord blood bilirubin seen as they are high risk groups.

Eldho et al in their study in 120 healthy term neonates concluded cord bilirubin level of 3.5 mg/dl, hyperbilirubinemia can be predicted with

sensitivity of 38.4%, specificity of 90.4% and positive predictive value of 52.6% and negative predictive value of 84.1%(35) [15].CBB values are comparable to findings of our study and similar to our study they only included term healthy babies born to cesarean sections. In contrast to our study this study had lower sensitivity and specificity with lower PPV and NPV.

Similarly, Bhat et al. on their study on 300 newborns found that 11% of them developed significant jaundice and cord blood bilirubin > 3 mg/dL and albumin less than 2.4 gm/dL are predictors of significant jaundice requiring treatment. [16] The incidence of significant jaundice was (11%) comparable to our study.in contrast to our study the sample size was bigger and also they included parameters like cord albumin, bilirubin – albumin ratio and the Cord blood albumin was found to be best predictive value, followed by bilirubin/albumin ratio and cord blood bilirubin in predicting development of subsequent neonatal hyperbilirubinemia. These parameters were not included in our study.

Similarly, Gupta et al. on their study on 152 healthy term newborns found that 15.5% of them developed significant jaundice. The cut-off values obtained for albumin and bilirubin of cord blood was < 2.56 mg/dL and >2.33 mg/dL.[17] At this cut-off the sensitivity of the test was found to be 94.12%, specificity was 86.67%, positive predictive value was 47.06%, negative predictive value was 99.15% and diagnostic accuracy was 87.50%. In contrary to our study cord blood albumin was used however, the predictive value of cord blood bilirubin was found to be greater than the predictive value of albumin in cord blood, with higher sensitivity and specificity being obtained on the ROC curve analysis at the cut-off point and larger area under curve.

Haridas et al. in their study on 500 healthy neonates found that umbilical cord blood bilirubin level > 1.78 gm/dL was 90% sensitive and 87% specific with a PPV of 75% and NPV of 92% in predicting significant neonatal hyperbilirubinemia. The results are different from our study as in our study positive predictive value is quite low and bilirubin level 2.95 is significant.[18]

The study done by Janaki et al in 2018, showed the cut-off value of umbilical cord bilirubin for development of significant hyperbilirubinemia for the study population was 1.85 mg/dl. This value predicts the development of significant hyperbilirubinemia with a sensitivity of 70.6% and specificity of 82.7%.[19]

Ruchika et al in their study in 240 healthy term neonates concluded that cord blood total bilirubin cut off value of 1.79mg/dl had sensitivity (82.5%), specificity (55.5 %), PPV (27.04%) and NPV (94.06) and can be used as a good predictor.[20]

Table 7: Comparison of cut-off values of umbilical cord bilirubin with various studies to predict neonatal hyperbilirubinemia.

AUTHOR	YEAR	CBB values used to predict NH
Naharb Z et al[11]	2009	2.5
Eldho et al[15]	2022	3.5
Bhat et al[16]	2019	3.0
Gupta et al[17]	2018	2.33
Haridas et al[18]	2018	1.78
Janaki et al[19]	2018	1.85
Ruchika et al[20]	2019	1.79

Other studies also reported the relation between raising levels of cord bilirubin and increased incidence of significant hyperbilirubinemia. Thus, from the above table it can be seen that different authors have used different cut off value for predicting significant hyperbilirubinemia.

Azma et al in 2011, have found that CBB cannot be used as a prediction of subsequent hyperbilirubinemia

These different values of cord blood bilirubin from different studies is mainly because of technical error in estimating bilirubin levels, sample size, cut off value decided for significant hyperbilirubinemia, as many studies were done before the Bhutani hour specific nomogram were introduced. Hence the need for a local laboratory to define the cut off value becomes all the more important.

It has always been a subject of discussion about the length of stay of the newborn mother in the institution, safety and danger of before time hospital discharge (jaundice, feeding problem, screening, anomaly detection) and designing a follow-up plan for each nation considering its economic constraint, community attitude, health infrastructure etc. Neonatal hyperbilirubinemia has been found to be the most prevalent reason for readmission of the newborns. The most recent guideline of AAP - states that infants discharged before 24 hours of age should be seen at 72 hours of age, discharged between 24 - 48 hours of age at 96 hours of age, and those discharged between 48 and72 hours of age should be seen at 5th day of life. However, in our country a complete follow up is not always possible because of noncompliance and lack of medical facilities in peripheral areas.

Thus, there is utmost need for framing up our own discharge policy and follow up program, since neonatal hyperbilirubinemia can be treated easily. Identifying high risk newborn and ensuring their follow up or delaying discharge is very important.

Our study population consisted of healthy term babies not in high-risk group. This is most important point of our study. And we also found use of cord blood bilirubin to be important for ruling out risk of jaundice.

Classifying newborns at low risk for hyperbilirubinemia minimize unnecessary prolongation of hospitalization as it will provide more confidence to pediatricians in decision of early discharge. Babies with high-risk category can be asked for early follow-up or parents can be counselled for need of delayed discharge if timely follow up cannot be ensured. So that simple, safe and economic phototherapy as a treatment option can be provided to reduce neonatal morbidity and mortality.

Our study had been conducted in O positive mothers undergoing cesarean section and we have not included Normal delivery babies (due to early discharge as well as inability to follow them till 72 hours of life). However, the results from this study can be used to predict risk of developing hyperbilirubinemia even in babies born to vaginal deliveries and may prevent further morbidity and mortality.

Such a proposal may therefore constitute an additional predictive method that is available for evaluating the occurrence of severe hyperbilirubinemia by third day of life. In association with other resources that were already available this proposal may help in assuring safer early discharge. Even in resource limited rural setups where there are limited resources and few hospital beds. Healthy newborns at low risk for hyperbilirubinemia can be discharged early from the hospital.

Based on the results obtained, we recommend for large sized studies to be carried out so that an in-depth information regarding the relationship between raised cord blood bilirubin with neonatal jaundice could be established.

The study was done in single center with limited sample size, which is a major limitation of study (small sample size). only full-term healthy neonates were taken for study. Given early discharge, vaginally delivered babies were excluded. This study has not considered the length of stay of neonates, and associated morbidity and mortality have not been discussed.

CONCLUSIONS

From the results and observations of the present study, we found that cord blood bilirubin levels over 2.95 mg/dL can be used as a predictive index for the development of significant jaundice. Babies with cord blood total bilirubin >2.95 mg/dl should be followed up closely to

prevent the severe consequences of neonatal hyperbilirubinemia like kernicterus. In comparison, those babies with cord total bilirubin <2.95 mg/dl can be safely discharged early to minimize unnecessary prolongation of hospitalization.

The umbilical cord blood is an essential predictor for newborns' subsequent occurrence of significant hyperbilirubinemia. We, however, suggest that large-scale studies be carried out so that in-depth information regarding the relationship between raised cord blood bilirubin and neonatal jaundice can be established.

Conflict of interest: None.

NJHS

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