## **Original Research Article**

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# Prediction of significant hyperbilirubinemia by estimating cord blood bilirubin in neonates with ABO incompatibility

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## **ABSTRACT**

**Background:** Neonatal hyperbilirubinemia is a common condition that may occur in 60-70 % of term babies and the most common pathological cause leading to hyperbilirubinemia is ABO incompatibility. ABO incompatible newborns are reported to be at greater risk of significant hyperbilirubinemia and are associated with significant morbidity like development of kernicterus. So early intervention, at proper time, is mandatory to prevent this sequel.

Methods: A prospective study included 240 term new-borns with gestational age of >37 weeks and birth weight >2.5 kg born to 'O' blood group mothers. Soon after delivery, cord blood was sent for blood group, total bilirubin and direct coombs test. All enrolled new-borns were assessed clinically daily till day 5th for appearance of hyperbilirubinemia using Kramer method. The data was analysed using descriptive analysis, ROC curve in SPSS version 14.

Results: Among two hundred forty ABO incompatible newborns, 40 (17%) had developed hyperbilirubinemia and among them most common blood group associated was B+ve (75%). Association between cord bilirubin content and maximum serum bilirubin content among newborn who developed hyperbilirubinemia was found to be significant (P value <0.01). As per ROC curve analysis, 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).

**Conclusions:** Cord blood total bilirubin levels ≥1.79mg/dl has a good predictive ability for prediction of significant hyperbilirubinemia among ABO incompatible new-born. DCT is neither specific nor sensitive screening tool for development of Neonatal hyperbilirubinemia in ABO incompatibility.

Keywords: Cord blood, Hyperbilirubinemia, Neonatal

#### INTRODUCTION

Hyperbilirubinemia is a common problem in newborns requiring medical attention. It occurs in 60 % term and 80 % preterm newborns during first seven days after birth.<sup>1</sup> Early discharge of healthy term newborns has become a common practice because of medical reasons like prevention of nosocomial infections and financial limitations. Thus, the recognition, follow up and early treatment of jaundice has become more difficult.<sup>2</sup> American Academy of Pediatrics recommends that newborn discharged within 48 hours should have a follow up visit after 48 to 72 hours for any significant jaundice and other problems.<sup>3</sup> One of the most common cause for readmission during the early neonatal period is neonatal hyperbilirubinemia. Significant hyperbilirubinemia occurs in 5-10% healthy term infants.<sup>4</sup> Pathologic levels of bilirubin may lead to irreversible complications such as bilirubin encephalopathy called kernicterus associated with cerebral palsy, deafness, dental dysplasia, ophthalmoplegia with upward gaze, and personality disorders.5

With declining incidence of Rhesus disease (Rh disease), as it is preventable by use of prophylactic anti-D, ABO incompatibility is the commonest cause of hemolytic jaundice in the newborns. Approximately 20% of all pregnancies are associated with ABO incompatibility between mother and the foetus and only <10% of all these cases manifests ABO-hemolytic diseases of the newborn (ABO-HDN).<sup>6</sup>

ABO incompatibility occurs in 'A', 'B' and 'AB' blood group babies born to 'O' blood group mothers. In mother with type A and type B blood group, antibodies of IgM class are present which do not cross placenta where as in type O blood group mother, antibodies are of IgG class which crosses the placenta. Low antigenicity of A and B factors and wide expression in variety of tissues beside RBCs, accounts for relatively low incidence and milder nature of ABO haemolytic disease.<sup>7</sup>

The diagnosis of ABO incompatibility is suspected by early onset of jaundice which usually appears during first 24 to 72 hours of life, mild splenomegaly and anaemia being rare manifestations whereas hydrops fetalis is extremely rare. Though a positive coombs's test is more often found in those babies with moderate and severe hyperbilirubinemia, the reaction is often weak and there is an unacceptably high number of 'false negative' results. 9

An association between cord bilirubin levels and subsequent risk of developing significant hyperbilirubinemia has been reported. 4,10,11 This information has prompted a more aggressive approach to early management of neonatal jaundice aimed at prevention of irreversible complication.

Studies of various strategies of predicting and dealing with neonatal hyperbilirubinemia are present in literature which includes follow up of infants discharged at or before 48 hours, pre-discharge bilirubin measurement, clinical assessment of risk factors for development of jaundice and routine transcutaneous bilirubin measurement. These recommendations have limited practical applicability in resource limited countries such as India. Availability of simple, non-invasive, economical method to predict significant hyperbilirubinemia can help clinicians in early discharge of children and selective follow up of high-risk infants. This study was conducted to determine incidence of ABO incompatibility, ABO iso immune disease in newborn and determination of cord bilirubin level to predict significant hyperbilirubinemia.

#### **METHODS**

This was a prospective study which included 240 consecutive healthy term babies born to O<sup>+</sup>ve mothers. This study was carried out at the tertiary care center in Rajasthan India. Study was conducted during one-year period, after obtaining permission from ethical committee of institute.

#### Inclusion criteria

 Only full-term babies (gestational age>37 weeks, determined by LMP and confirmed by new Ballard score) whose parents were willing for further follow up were included in the study.

#### Exclusion criteria

• Babies with rhesus blood factor incompatibility, Significant illness requiring intensive care management for more than 12 hours, requiring resuscitation (positive pressure ventilation) at birth, excessive weight loss (>10%), cephalohematoma, major congenital malformations, Infant of diabetic mother were excluded from the study.

Hyperbilirubinemia was defined as total serum bilirubin level >12mg/dl at 25 to 48 hours of life, >15mg/dl between 49 to 72 hours and >17mg/dl beyond 72 hours of life. Cord blood was collected for estimating blood group, bilirubin and DCT for all babies born to O<sup>+</sup>ve blood groups. The study population was initially followed up for first 5 days of life clinically by Kramer's method <sup>12</sup> Newborns identified with jaundice were followed up. using serial serum bilirubin values. Two ml of venous blood sample was collected in plain vial from the baby on day 5<sup>th</sup> of life or earlier if clinically indicated. Serum bilirubin was estimated using diazo method. <sup>13</sup>

Sensitivity, specificity and positive and negative predictive values of different cut off points of cord bilirubin were obtained and receiver operating characteristic (ROC) analysis was carried out to elucidate a cut off value for which sensitivity, specificity, positive predictive value, negative predictive value was presented in its percentage.

## Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for MS Windows. The obtained data was entered into MS Excel. The data was analysed using descriptive statistics, chi square.

## **RESULTS**

A total of 240 healthy full-term ABO incompatible newborns were studied. Those who have developed significant hyperbilirubinemia were classified as hyperbilirubinemia and remaining babies were observed as non-hyperbilirubinemia. Various analysis and interpretation were made by comparing the two groups.

Out of 240 newborns, 40 (17%) newborns developed jaundice and were classified as significant hyperbilirubinemia and 200 (83%) who did not developed jaundice were grouped as non-hyperbilirubinemia. Among 40 newborns who developed significant hyperbilirubinemia, 63 % newborns were

from 2.5-3.00kg birth weight (p value 0.550). Most common blood group associated with ABO incompatibility leading to significant hyperbilirubinemia was B+(75%) followed by A+(22%) and AB+(3%) (p value <0.01). 70% newborns who develop significant hyperbilirubinemia were early term (37-38 weeks) (p

value <0.05). 60 % newborns who developed significant hyperbilirubinemia were delivered by LSCS (p value 0.7705). There was no significant difference in the number of male (55%) and female (41 %) babies developing significant hyperbilirubinemia (p value <0.750) (Table 1).

Table 1: Baseline characteristics between significant hyperbilirubinemia new-borns and non-hyperbilirubinemia new-borns.

Characteristics	Non-hyperbilirubinemia Number (%)	Significant hyperbilirubinemia Number (%)	P value	
Birth weight				
2.5-3.00kg	108 (54)	25 (63)	< 0.550	
3.01-3.50kg	49 (25)	9 (22)	<0.550	
3.50kg and above	43 (21)	6 (15)		
Baby blood group				
A+	69 (35)	9 (22)		
B+	111 (55)	30 (75)	< 0.01	
AB+	20 (10)	1 (3)	<0.01	
Gestational age				
37-38 weeks	133 (66)	28 (70)	< 0.05	
39-40 weeks	67 (34)	12 (30)	<0.03	
Mode of delivery				
NVD	85 (42)	16 (40)	0.7705	
LSCS	115 (58)	24 (60)	0.7703	
Gender				
Male	102 (51)	22 (55)	< 0.750	
Female	98 (49)	18 (41)	<0.730	

Mean cord bilirubin of newborns who developed significant hyperbilirubinemia was 2.23 with SD of 0.70. Significant hyperbilirubinemic newborns were distributed according to development of hyperbilirubinemia as before 5<sup>th</sup> day (clinically observed) 26 (65 %) and on day 5th 14 (35%). Mean serum bilirubin of newborns who developed significant hyperbilirubinemia was 15.58 with SD of 2.09.

Table 2: Association between cord bilirubin content and maximum serum bilirubin content among nonhyperbilirubinemia newborns and significant hyperbilirubinemia new-borns.

	Non -hyper- bilirubinemia (200)		Significant hyper-bilirubinemia (40)	
	Mean	SD	Mean	SD
Serum cord bilirubin	1.74	0.55	2.23	0.70
Maximum serum bilirubin	9.64	1.14	15.58	2.09
P-value	< 0.01			

Mean cord bilirubin and maximum serum bilirubin was higher in newborns who developed significant

hyperbilirubinemia and the difference was statistically highly significant with p value of <0.01 (Table 2).

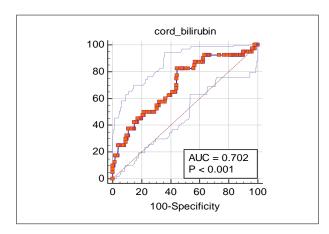


Figure 1: ROC curve for cut off value of cord bilirubin.

Out of 240 newborns, 4 newborns were DCT positive and 236 were DCT negative. Mean cord bilirubin in DCT positive and DCT negative newborns were 1.5±0.33 and 1.83±0.61 respectively which was statistically not significant. Maximum serum bilirubin was higher in DCT

positive (11.37±2.06) than DCT negative (10.57±2.48) newborns and this difference was statistically significant. Among DCT+ newborns, phototherapy was received by 25% newborns and among DCT-newborn, phototherapy was received by 16.52%. This association between hyperbilirubinemia in DCT positive, DCT negative newborns and phototherapy was statistically not significant (p value <0.965) (Table 3).

Receiver operating characteristic (ROC) curve analysis was used to determine the cut off value of serum cord

bilirubin which would predict neonates likely to develop significant hyperbilirubinemia. (Figure 1).

The cut off value of 1.79 mg/dl had sensitivity (82.5%), specificity (55.5 %), PPV (27.04%) and NPV (94.06%).

Among 40 newborns who developed significant hyperbilirubinemia, 33 newborns were above the cut off value of 1.79 mg/dl and 7 newborns were below 1.79 mg/dl. Association between bilirubin contents was observed to be significant (Table 4).

Table 3: Correlation between hyperbilirubinemia and DCT positive and negative new-borns.

Baseline characteristics	DCT (+ve) (No. of new-borns)	DCT (-ve) (No. of new-borns)	p value
Cord bilirubin content (Mean±SD)	1.5±0.33 (4)	1.83±0.61 (236)	0.282
Maximum serum bilirubin (Mean±SD)	11.37±2.06 (4)	10.57±2.48 (236)	< 0.01
Phototherapy	25%	16.52%	< 0.965

Table 4: Predictive value of cord bilirubin for significant hyperbilirubinemia.

Cord bilirubin level	Significant hyper- bilirubinemia	Non- hyperbilirubinemia	Sensitivity	Specificity	PPV	NPV
≥1.79	33	89				
≤1.79	7	111	82.5%	55.5%	27.04%	94.06%
Total	40	200				

#### **DISCUSSION**

Among 240 newborns enrolled, 40 (17%) newborns developed significant hyperbilirubinemia. Incidence of neonatal hyperbilirubinemia in present study is higher than earlier studies. Heier et al, in their study on maternal blood group O+ve as a risk factor of neonatal hyperbilirubinemia requiring treatment had found that ABO incompatible babies born to O+ mothers had the double risk to develop jaundice requiring treatment.14 Various other studies also show ABO incompatibility as an important cause of neonatal jaundice. 15,16 In the present study, 40 newborns who develop significant hyperbilirubinemia, 63 % were between 2.5-3.00kg, 22% were between 3.01-3.50kg and 15% were 3.50kg and above which was not statistically significant (p < 0.550) (Table 1). 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.17,18

In present study, results showed that blood group incompatibility between O<sup>+</sup>ve mothers and OA and OB blood group of newborns leads significant hyperbilirubinemia (Table 1). ABO blood group heterospecific (mother group O, newborn A or B) newborns are at risk for hyperbilirubinemia due to immune based haemolysis (Murray and Roberts, 2007). <sup>19</sup>

Blood group incompatibility is associated with increased incidence of hyperbilirubinemia Ahire et al.  $^{20}$  Most common blood group associated with neonatal hyperbilirubinemia was B+ve (75%) followed by A+ve (33%) and AB+ve (8%) (P value <0.001).

In the present study, significant hyperbilirubinemia was observed in 70 % newborns belonged to 37-38 weeks and 30% in >38 weeks gestational age which was statistically significant (p value <0.05) (Table 1). It indicates that hyperbilirubinemia is more common in early term newborns (37-38 weeks). These results are similar to other studies documented such as Singhal et al, (16.7%) Narang et al, (47.9%) in which gestational age was observed to have significant effect on hyperbilirubinemia. 4.21

In the present study, significant neonatal hyperbilirubinemia was found in 42% of NVD and 58% of LSCS. Although bilirubin content was higher in newborn delivered via LSCS, but no significant relationship was found between mode of delivery and neonatal hyperbilirubinemia. (p<0.678) (Table 1) This implies that neonatal hyperbilirubinemia is independent of mode of delivery. There are several reports available suggested no influence of mode of delivery on newborns bilirubin level. Boskabadi et al, found no significant relationship between the mode of delivery and the incidence of jaundice.<sup>22</sup> Unnecessary interventions during delivery such as excessive use of oxytocin during labour and caesarean section are also considered as the risk factors Tamook et al.<sup>23</sup> According to a study by Tamook et al, the prevalence of jaundice was higher among neonates born by caesarean section, compared to those who were naturally delivered. On the contrary, Chang et al, reported that bilirubin level was higher among naturally delivered neonates, compared to those born by caesarean section.<sup>24</sup>

In the present study, 55% were male and 41% were significant female who developed neonatal hyperbilirubinemia, association of which was statistically not significant (p <0.750) (Table 1). It can be inferred that the bilirubin level is independent of sex of the newborn. This was supported by study done by Banasia et.al, on contrary Satrya et al. <sup>25,26</sup> showed that male sex more risk of readmission for neonatal has hyperbilirubinemia. This could be explained on the basis that in developing countries male neonates are given more care as compared to the female neonates because of gender discrimination prevalent in society of the developing countries.

In the present study, association between cord bilirubin content and maximum serum bilirubin content among newborn who developed significant hyperbilirubinemia was found to be significant (Table 2). This was supported by study done by Knupfer et al, in which they concluded that umbilical cord serum bilirubin is useful in predicting the postnatal bilirubin values in term and near-term newborns.<sup>27</sup> In present study, 4 newborns were DCT positive and rest 236 DCT negative. Association between significant hyperbilirubinemia and cord bilirubin content in DCT positive and negative newborns were compared and found to be non-significant in both groups. Association between significant hyperbilirubinemia and maximum serum bilirubin content in DCT positive was higher as compared to DCT negative newborns and this association was found to be significant (Table 3). Thus, it can be inferred that DCT was not sensitive to predict neonatal hyperbilirubinemia. Among 4 newborns who were DCT positive, only 1 required phototherapy, suggesting low specificity of DCT. Hence, in present study, DCT is neither specific nor sensitive screening tool for development of neonatal hyperbilirubinemia in ABO incompatibility. Similar findings were suggested by Mazzi R et al, Pradhan et al, that DCT is not by itself a reliable method of predicting the severity hyperbilirubinemia. 28,29

In the present study, cord bilirubin content with cut off value of 1.79 mg/dl as per ROC analysis, had sensitivity (82.5%), specificity (55.5%), PPV (27.04%) and NPV (94.06%) (Figure 1). It can be concluded that bilirubin level that were equal to or greater than 1.79 mg/dl of cord bilirubin content can be considered as good predictor of significant hyperbilirubinemia with high negative predictive values and high level of sensitivity and specificity. However, the cord bilirubin level of <1.79 did

not completely exclude the development of significant hyperbilirubinemia. 94.06% negative predictive value in the present study suggested that measurement of cord serum bilirubin can help in identify those newborns that are unlikely to require further evaluation and intervention. Rudy et al, determined this value using ROC as 2.54mg/dL having high sensitivity and specificity.<sup>30</sup> Amar et al, value was more than 2 mg/dL which had highest specificity and this critical bilirubin level had a very high NPV and fairly low PPV.<sup>31</sup>

#### **CONCLUSION**

This study concludes that there is a significant relationship development between the hyperbilirubinemia in neonates and cord blood total bilirubin levels among ABO incompatible new-born. Cord blood total bilirubin levels ≥1.79mg/dl has a good predictive ability to predict the occurrence of hyperbilirubinemia among ABO incompatible new-born. In present study, DCT is neither specific nor sensitive screening tool for development of Neonatal hyperbilirubinemia in ABO incompatibility. In addition, results showed that factors like mode of delivery, baby birth weight and gender does not have significant effect on neonatal hyperbilirubinemia. In present study, it was concluded that significant hyperbilirubinemia is more common in early term (37-38 weeks) new-borns. Blood group incompatibility as a whole is associated with increased incidence of hyperbilirubinemia. In present study new-born with blood group B+ve are most likely to develop neonatal hyperbilirubinemia among ABO incompatible new-borns.

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Institutional Ethics Committee

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