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Original research article

To determine the cord blood nucleated RBCs as a potential tool in prediction of neonatal hyperbilirubinemia in ABO incompatibility susceptible neonates: prospective study

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Abstract

Aim: to determine the cord blood nucleated RBCs as a potential tool in prediction of neonatal hyperbilirubinemia in ABO incompatibility susceptible neonates.

Material and methods: This prospective study was done the Upgraded Department of Paediatrics, Patna Medical College & Hospital, Patna, Bihar, India, from August 2015 to November 2016. Total 120 consecutive healthy term babies born to O^+ve mothers were included in this study. Cord blood was collected for estimating blood group, bilirubin and DCT for all babies born to O^+ve blood groups. The study population was initially followed up for first 5 days of life clinically by Kramer's method. 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^{th} of life or earlier if clinically indicated. Serum bilirubin was estimated using diazo method. **Results:** Out of 120 newborns, 20 (16.67%) newborns developed jaundice and were classified as significant hyperbilirubinemia and 100 (83.33%) who did not developed jaundice were grouped as non- hyperbilirubinemia. Among 20 newborns who developed significant hyperbilirubinemia, 65 % newborns were from 2.5-3.00kg birth weight (p value 0.529). Most common blood group associated with ABO incompatibility leading to significant hyperbilirubinemia was B+ (80 %) followed by A+ (15 %) and AB + (5%) (p value <0.01). 75% newborns who develop significant hyperbilirubinemia were early term (37-38 weeks) (p value <0.05). 55 % newborns who developed significant hyperbilirubinemia were delivered by LSCS (p value 0.84). There was no significant difference in the number of male (60%) and female (40 %) babies developing significant hyperbilirubinemia (p value <0.81). Mean cord bilirubin of newborns that developed significant hyperbilirubinemia was 2.27 with SD of 0.68. Significant hyperbilirubinemic newborns were distributed according to development of hyperbilirubinemia as before 5^{th} day (clinically observed) 12 (60 %) and on day 5th 8 (40%). **Conclusion:** 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

Neonatal hyperbilirubinemia is mostly benign and resolve unprompted. But it can lead to dreadful complications like kernicterus which can ultimately result in significant morbidity.[1] There are several causes for indirect hyperbilirubinemia such as escalated enter hepatic

circulation, reduced conjugation of bilirubin, defective hepatic uptake and increased production of bilirubin due to hemolysis.[2,3] Although Rh incompatibility comprise the majority of hemolytic disease of newborn, incompatibility of ABO blood group is not rare. ABO incompatibility is seen when a mother with O blood group carries a fetus with a different blood type (type A, B, or AB).[4] Due to brief period of postpartum stay in hospital, the identification and timely management of this condition is of prime importance.[5] Even the healthy term neonates are readmitted after discharge due to neonatal hyperbilirubinemia.[6,7] Nucleated RBCs are the predecessors of RBC which are seen in peripheral circulation in newborn period.[8,9] The number of nRBCs in peripheral smear can vary in different conditions.[8] Some conditions like birth asphyxia, blood loss/hemolysis, maternal diabetes, intrauterine infections, intrauterine growth restriction and preeclampsia can cause surge in the nucleated RBCs into circulation.[10] The pro-oxidant nature of bilirubin is dreadful than its antioxidant property.[11] This study is taken up to study the importance of cord blood nucleated RBCs in neonatal hyperbilirubinemia. Bilirubin is an important antioxidant at physiological limits, but acts as a stronger pro-oxidant at higher levels. It is generated by the degradation of heme, whose source is red blood cells. The investigation of relation between hyperbilirubinemia and the increase of nRBCs in perinatal problems may be therefore of great importance. the aim of the presents study was to determine the cord blood nucleated RBCs as a potential tool in prediction of neonatal hyperbilirubinemia in ABO incompatibility susceptible neonates.

Material and methods

This prospective study was done the Upgraded Department of Paediatrics, Patna Medical College & Hospital, Patna, Bihar, India, from August 2015 to November 2016, after taking the approval of the protocol review committee and institutional ethics committee.

Total 120 consecutive healthy term babies born to O^+ve mothers.

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.

Methodology

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^+ve blood groups. The study population was initially followed up for first 5 days of life clinically by Kramer's method 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^{th} of life or earlier if clinically indicated. Serum bilirubin was estimated using diazo method.

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.

Results

A total of 120 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 120 newborns, 20 (16.67%) newborns developed jaundice and were classified as significant hyperbilirubinemia and 100 (83.33%) who did not developed jaundice were grouped as non- hyperbilirubinemia. Among 20 newborns who developed significant hyperbilirubinemia, 65 % newborns were from 2.5-3.00kg birth weight (p value 0.529). Most common blood group associated with ABO incompatibility leading to significant hyperbilirubinemia was B+ (80 %) followed by A+ (15 %) and AB + (5%) (p value <0.01). 75% newborns who develop significant hyperbilirubinemia were early term (37-38 weeks) (p value <0.05). 55 % newborns developed significant hyperbilirubinemia were delivered by LSCS (p value 0.84). There was no significant difference in the number of male (60%) and female (40 %) babies developing significant hyperbilirubinemia (p value <0.81) (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	52 (52)	13 (65)	<0.529
3.01-3.50kg	26 (26)	4 (20)	
3.50kg and above	22 (22)	3 (15)	
Baby blood group			
A+	36 (36)	3 (15)	
B+	52 (52)	16 (80)	<0.01
AB+	12 (12)	1 (5)	
Gestational age			
37-38 weeks	65 (65)	15 (75)	<0.05
39-40 weeks	35 (35)	5 (25)	
Mode of delivery			
NVD	39 (39)	9 (45)	0.84
LSCS	61 (61)	11 (55)	
Gender			
Male	52 (52)	12(60)	<0.81
Female	48 (48)	8 (40)	

Mean cord bilirubin of newborns who developed significant hyperbilirubinemia was 2.27 with SD of 0.68. Significant hyperbilirubinemic newborns were distributed according to development of hyperbilirubinemia as before 5^{th} day (clinically observed) 12 (60 %) and on day 5th 8 (40%). Mean serum bilirubin of newborns who developed significant hyperbilirubinemia was 16.17 with SD of 2.21.

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

	Non -hyper- bilirubinemia (200)		Significant hyper-bilirubinemia (40)	
	Mean	**SD**	**Mean**	**SD**
Serum cord bilirubin	1.69	0.62	2.27	0.68
Maximum serum bilirubin	9.47	1.21	16.17	2.21
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).

Out of 120 newborns, 3 newborns were DCT positive and 117 were DCT negative. Mean cord bilirubin in DCT positive and DCT negative newborns were 1.6±0.27 and 1.77±0.59 respectively which was statistically not significant. Maximum serum bilirubin was higher in DCT positive (12.07±2.14) than DCT negative (10.88±2.57) newborns and this difference was statistically significant. Among DCT+ newborns, phototherapy was received by 20% newborns and among DCT-newborn, phototherapy was received by 15%. This association between hyperbilirubinemia in DCT positive, DCT negative newborns and phototherapy was statistically not significant (p value <0.912) (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. The cut off value of 1.79 mg/dl had sensitivity (81.5%), specificity (56 %), PPV (28.5%) and NPV (94%).

Among 20 newborns developed significant hyperbilirubinemia, 17 newborns were above the cut off value of 1.79 mg/dl and 3 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)	1.6±0.27 (3)	1.77±0.59 (117)	0.267
Maximum serum bilirubin (Mean±SD)	12.07±2.14 (3)	10.88±2.58 (117)	<0.01
Phototherapy	20%	15%	<0.912

Table 4: Predictive value of cord bilirubin for significant hyperbilirubinemia

Cord bilirubin level	Significant hyper-bilirubinemia	Non-hyperbilirubinemia	Sensitivity	Specificity	PPV	NPV
≥1.79	17	47	81.5%	56%	28.5%	94%
≤1.79	3	53				
Total	20	100				

Discussion

Among 120 newborns enrolled, 20 (16.67%) 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.[12]. Various other studies also show ABO incompatibility as an important cause of neonatal jaundice.[13,14] In the present study, 20 newborns who develop significant hyperbilirubinemia, 65 % were between 2.5-3.00kg, 20% were between 3.01-3.50kg and 15% were 3.50kg and above which was not statistically significant (p <0.529) 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.[15,16]

In present study, results showed that blood group incompatibility between O^+ve mothers and OA and OB blood group of newborns leads significant hyperbilirubinemia . 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).[17]

Blood group incompatibility is associated with increased incidence of hyperbilirubinemia Ahire et al.[18] Most common blood group associated with ABO incompatibility leading to significant hyperbilirubinemia was B+ (80 %) followed by A+ (15 %) and AB + (5%) (p value <0.01).

In the present study, significant hyperbilirubinemia was observed in 75 % newborns belonged to 37-38 weeks and 25% in >38 weeks gestational age which was statistically significant (p value <0.05). 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.[19,20]

In the present study, significant neonatal hyperbilirubinemia was found in 45% of NVD and 55% 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<0.84). 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.[21] 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.[22] 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.[23]

In the present study, 60% were male and 40% were female who developed significant neonatal hyperbilirubinemia, association of which was statistically not significant (p <0.81). 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.[24,25] showed that male sex has more risk of readmission for neonatal 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. 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.[26] In present study, 3 newborns were DCT positive and rest 117 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. Thus, it can be inferred that DCT was not sensitive to predict neonatal hyperbilirubinemia. Among 3 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 of hyperbilirubinemia.[27,28]

In the present study, cord bilirubin content with cut off value of 1.79 mg/dl as per ROC analysis, had sensitivity (81.5%), specificity (56%), PPV (28.5%) and NPV (94%). 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% 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.[29] 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.[30]

Conclusion

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.

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