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Original Article

Paediatrics Section

Predictability of Umbilical Cord Serum Bilirubin in Estimating the Risk of Hyperbilirubinemia in Term Neonates with ABO Incompatibility- A Prospective Cohort Study

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ABSTRACT

Introduction: Neonatal hyperbilirubinemia is a significant cause of morbidity among term neonates. Delayed follow-up of newborns may lead to considerable morbidity and encephalopathy. High bilirubin levels may necessitate exchange transfusion, especially in infants with ABO or Rh blood incompatibility. Measurement of Umbilical Cord Bilirubin (UCB) is an easily available, inexpensive and non invasive method to predict the development of hyperbilirubinemia in newborns.

Aim: To determine the predictive value of UCB in estimating the risk of subsequent hyperbilirubinemia in term neonates with ABO incompatibility and to establish a UCB cut-off associated with the risk of hyperbilirubinemia development.

Materials and Methods: This prospective cohort study was conducted at the Punjab Institute of Medical Sciences (PIMS) in Jalandhar, Punjab, India, from June 2022 to March 2023. A total of 113 term babies born to O+ mothers were included in the study. At the time of delivery, cord blood was drawn from the placental side of the cord for blood group, Direct Coomb's Test (DCT), and serum bilirubin analysis. Infants were followed-up every 12 hours while inpatient and every 48 hours after discharge until day 5 for assessment of clinical jaundice. Significant hyperbilirubinemia was managed with phototherapy according to American Academy of Paediatrics (AAP) guidelines. The data was collected and subjected to statistical analysis using IBM Statistical Package for Social Sciences (SPSS) 20.0 software, with a significance level set at p-value <0.05, utilising Receiver Operating Characteristic (ROC) curve analysis.

Results: A total of 113 term neonates were enrolled in the study, of which 57 (50.4%) were females. The mean cord bilirubin level was 2.6±1.06 mg/dL. A total of 40 (35.3%) babies developed significant jaundice requiring phototherapy, with a mean highest bilirubin level of 15±3.2 mg/dL. Using a cord bilirubin level of ≥2.0 mg/dL, significant hyperbilirubinemia could be predicted with a sensitivity of 80%, specificity of 54%, positive predictive value of 57.97%, and negative predictive value of 75%. There was a positive correlation between cord bilirubin and postnatal serum bilirubin in the present study, as determined by ROC analysis {Area Under Curve (AUC) >0.5}.

Conclusion: The present study concluded that UCB is an important predictor of significant hyperbilirubinemia occurrence in newborns with ABO incompatibility.

Keywords: ABO mismatch, Blood incompatibility, Jaundice, Newborns, Postnatal complication

INTRODUCTION

Hyperbilirubinemia is a commonly observed complication in neonates [1]. It is one of the most prevalent reasons for the readmission of neonates to the hospital [2]. Hyperbilirubinemia is found in 80% of preterm and 60% of term newborns [3]. More than 80% of newborns usually exhibit some degree of visible jaundice upon follow-up [4]. It is essential to carefully monitor their bilirubin levels and treat them accordingly because, in severe cases, unconjugated bilirubin can deposit within the brain, mainly in the basal ganglia, leading to kernicterus [5]. Effective early management can prevent these serious complications.

Clinically significant jaundice may not develop until three days after delivery [6]. Nowadays, it is common practice to discharge healthy term newborns early for social, financial, and medical reasons. As a result, it has become difficult to diagnose and treat jaundice in neonates [7].

The AAP advises that newborns discharged within two days of delivery should be followed-up after 48-72 hours to assess the occurrence of jaundice [8]. In developing countries like India, ensuring frequent follow-up visits is challenging due to poor compliance and lack of awareness [9]. Therefore, it is necessary to identify reliable predictive markers that can assist paediatricians in determining the incidence of hyperbilirubinemia.

Measurement of UCB is an easily available, inexpensive, and non invasive method to predict the development of hyperbilirubinemia among newborns [10]. It is already a standard practice to measure UCB for risk stratification of hyperbilirubinemia in cases of Rh incompatibility [4]. In addition to Rh Isoimmunisation, ABO incompatibility is an important cause of haemolytic jaundice among neonates. AAP guidelines also suggest that it is desirable to measure cord bilirubin in cases of mothers with O blood type [8].

Recently, many studies have evaluated the predictability of UCB in estimating the risk of subsequent hyperbilirubinemia in ABO incompatibility [11-16]. However, different studies have reported varying cut-offs ranging from 1.85 to 3.5 mg/dL [11-16]. Additionally, there is a paucity of research on this subject from North India.

Hence, the present study was planned to determine the predictability of UCB in estimating the risk of subsequent hyperbilirubinemia in term neonates with ABO incompatibility and to establish a UCB cut-off associated with the risk of hyperbilirubinemia development.

This would help screen babies with a high probability of developing neonatal jaundice in the future.

MATERIALS AND METHODS

The present prospective cohort study was conducted on all babies born to O+ mothers at term gestation in the Department of Paediatrics at Punjab Institute of Medical Sciences, Jalandhar, Punjab, India, from June 2022 to March 2023. The study was approved by the Institutional Research and Ethics Committee (vide letter no. PIMS/DP/Gen 163/1877-97).

Inclusion criteria: Mothers with a gestational age of more than 37 weeks and who have an O+ve blood group were included in the study after obtaining written informed consent. Inborn babies of O+ve mothers with blood groups A, B, or AB were enrolled.

Exclusion criteria: Babies with blood group O, those who were outborn, or those who suffered from concomitant Rh mismatch, G6PD deficiency, cephalhaematoma, or subgaleal bleed; infants of diabetic mothers; and babies with acidosis, asphyxia, or co-existing sepsis were excluded from the study. Mothers with a gestational age of less than 37 weeks were also excluded.

Sample size calculation: The sample size was calculated based on convenience sampling. All subjects fulfilling the inclusion criteria who presented during the study duration were enrolled.

Study Procedure

Case records of mothers meeting the inclusion criteria were assessed for the Period of Gestation (POG) and oxytocin use. The mode of delivery, gender of the baby, and birth weight were recorded. At the time of delivery, 5 mL of cord blood was drawn from the placental side of the umbilical cord for blood group, DCT, and serum bilirubin analysis. Babies were followed 12-hourly while inpatient and every 48 hours after discharge until day 5 for assessment of clinical jaundice. After discharge, babies were called for follow-up every 48 hours, and the paediatrician on duty examined the baby for visible icterus in broad daylight. Upon the appearance of clinically significant icterus, a 2 mL blood sample was drawn to estimate serum bilirubin levels. Significant hyperbilirubinemia was managed with phototherapy and/or exchange transfusion according to AAP guidelines [8]. For babies requiring phototherapy, a 2 mL blood sample was drawn in an Ethylenediamine Tetraacetic Acid (EDTA) vial and sent to the lab for estimation of haemoglobin, reticulocyte count, and qualitative G6PD analysis (quantitative analysis was not available in the study hospital). Babies with G6PD deficiency were labelled as "deficient" in the results. No additional intervention or follow-up was conducted in the study.

STATISTICAL ANALYSIS

The data were collected and subjected to statistical analysis using IBM Statistical Package for the Social Sciences (SPSS) 20.0 software, with a significance level set at p-value<0.05. Means and standard deviations were calculated for continuous variables, while frequencies and percentages were assessed and recorded for categorical variables. ROC curve analysis was used to determine the sensitivity and specificity of UCB cut-off values associated with significant hyperbilirubinemia. The correlation between UCB levels and the subsequent development of hyperbilirubinemia was estimated using ROC curve analysis, with an AUC greater than 0.5 considered significant.

RESULTS

A total of 139 mothers were screened for inclusion in the study. However, 19 babies were born with blood group O, one was the infant of a diabetic mother, two had subgaleal haemorrhage, two had birth asphyxia, one had sepsis, and one was found to be G6PD deficient. Consequently, these babies were excluded from the study. Thus, a total of 113 term neonates were included in the study, of which 57 (50.4%) were female. Notably, 70 (61.9%) were delivered by caesarean section. Among the 113 neonates, oxytocin was used in 64 (56.6%) cases. The blood group of the babies was assessed, with the majority, 62 (54.87%), being blood group B+, and 43 (38.05%) being blood group A+. Most cases, 110 (97.3%), were DCT negative, with only three being DCT positive. A total of 50 babies (44.2%) suffered from clinically significant jaundice, necessitating the estimation of total serum bilirubin, and 40 (35.4%) required phototherapy treatment. The remaining 10 clinically jaundiced babies, whose bilirubin levels were below the phototherapy cut-off, were followed-up every 48 hours until day 7 of life for any worsening of jaundice and the need for admission. A total of 19 (16.8%) neonates required readmission for the treatment of hyperbilirubinemia [Table/Fig-1].

Parameters		Frequency (n)	Percentage (%)
Gender	Female	57	50.4
	Male	56	49.6
MOD	LSCS	70	61.9
	NVD	43	38.1
Oxytocin use	No	49	43.4
	Yes	64	56.6
Baby blood group	B+	62	54.87
	B-	2	1.77
	A+	43	38.05
	A-	2	1.77
	AB+	4	3.54
DCT	Negative	110	97.3
	Positive	3	2.7
Jaundice	No	63	55.8%
	Yes	50	44.2%
	Significant jaundice*	40	35.4%
Phototherapy	Yes	40	35.4%
	No	73	64.6%
Readmission	No	94	83.2
	Yes	19	16.8
Total		113	100.0

[Table/Fig-1]: Baseline demographic data of newborns.
*Jaundice requiring phototherapy; MOD: Mode of delivery; LSCS: Lower segment caesarean section; NVD: Normal vaginal delivery; +: positive; -:negative; DCT: Direct coombs' test

The mean birth weight of the neonates was found to be 2.84±0.41 kg, and the mean POG was 38±0.96 weeks. The mean age at the onset of jaundice was 3±1.2 days, with the earliest onset being at 36 hours. Babies with jaundice requiring phototherapy belonged to a significantly lower gestational age group (p-value<0.05) and had slightly lower birth weights (p-value>0.05) compared to those without jaundice. Laboratory investigations revealed that the mean Cord Blood Bilirubin (CBB) level was 2.6±1.06 mg/dL. Babies with significant jaundice (those requiring phototherapy) had higher cord serum bilirubin levels (3.19±1.15 mg/dL) compared to those who did not have any jaundice (2.2±0.64 mg/dL). This difference was statistically significant (p=0.04) [Table/Fig-2].

Parameters	Total N=113 Mean±SD	With significant jaundice* n=40 Mean±SD	With jaundice not requiring phototherapy n=10 Mean±SD	Without jaundice n=63 Mean±SD	p-value
Mean POG (weeks)	38±0.96	38.5±0.89	38.3±0.49	38.9±0.92	0.03
Mean birth weight (kg)	2.84±0.41	2.8±0.37	2.8±0.6	2.87±0.41	0.37
Cord serum bilirubin (mg/dL)	2.6±1.06	3.19±1.15	2.6±1.1	2.2±0.64	0.04

[Table/Fig-2]: Mean of various baseline parameters.
*Jaundice requiring phototherapy POG: Period of gestation; SD: Standard deviation

Among the babies with significant jaundice, the mean haemoglobin level was 14.6±2.8 g/dL, the mean reticulocyte count was 2.3±1.2%, and the mean highest Total Serum Bilirubin (TSB) was 15±3.2 mg/dL, with the highest TSB value being 22.8 mg/dL [Table/Fig-3]. A total of 40 (35.4%) babies were treated with phototherapy according to AAP guidelines, and none required exchange transfusion. For the babies with jaundice, the mean duration of hospital stay was 4.56±1.2 days.

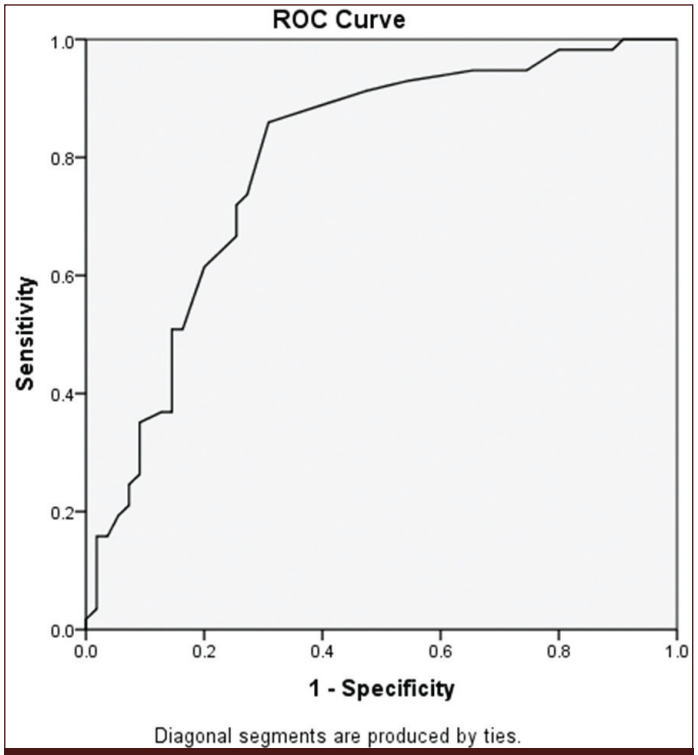
Investigations	Mean (n=40)	Standard deviation
Cord serum bilirubin (mg/dL)	2.6	1.06
Haemoglobin (g/dL)	14.6	2.8
Reticulocyte count (%)	2.3	1.2
Mean highest TSB (mg/dL)	15	3.2

[Table/Fig-3]: Results of laboratory investigations in babies with significant jaundice.
TSB: Total serum bilirubin

Using a cord bilirubin level of ≥2 mg/dL, significant hyperbilirubinemia could be predicted with a sensitivity of 80%, a specificity of 54%, a positive predictive value of 57.97%, and a negative predictive value of 75%. The cut-off cord bilirubin level determined for the present study population was 2.10 mg/dL. There was a positive correlation between cord bilirubin and postnatal serum bilirubin (p-value <0.05), with an AUC of 0.97 (>0.5) in the present study [Table/Fig-4,5].

Area under the curve				
Test result variable (s): Umbilical Cord Bilirubin (UCB)				
Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
0.970	0.021	0.002	0.930	1.000
Sensitivity	98.4%			
Specificity	85%			
Cut-off value	2.10			

[Table/Fig-4]: ROC curve for umbilical cord bilirubin.
a: standard error; b: significance



[Table/Fig-5]: ROC curve for Umbilical Cord Bilirubin (UCB).

DISCUSSION

The current study assessed 113 term neonates with ABO mismatch and found that the most common blood group among the neonates was B+ve. A total of 40 (35.4%) babies developed significant jaundice requiring phototherapy. The mean UCB was higher in babies requiring phototherapy compared to those without jaundice. According to the present study, UCB levels >2.1 mg/dL could predict subsequent neonatal hyperbilirubinemia with a sensitivity of 80% and a specificity of 54%.

Similar to the present study, Singh R and Jain H and Kumar TA et al., found that the most prevalent blood group linked with neonatal hyperbilirubinemia was B+ve (75% and 52%, respectively) [3,13]. In the present study, 50 (44.2%) cases with ABO mismatch developed clinically visible neonatal jaundice, while 40 (35.4%) required treatment with phototherapy. This is consistent with another study from North India by Bhat D and Purohit M, as well as a study from Tamil Nadu by Janaki AN and Selvakumar P, in which 33% and 24% of cases of ABO mismatch developed significant hyperbilirubinemia, respectively [12,15]. However, Thumjaa A and Vindhiya K and Kumar TA et al., reported a lower incidence of pathological jaundice (13% and 10%, respectively) [11,13]. This discrepancy may be explained by the higher use of oxytocin (56.6%) and a greater proportion of caesarean deliveries (61.9%) in our cohort. In accordance with the present study, Tamook A et al., stated that unnecessary interventions during delivery, such as excessive oxytocin use, are also risk factors for neonatal hyperbilirubinemia [17]. They found a higher prevalence of jaundice in neonates who were born by caesarean section compared to those who were delivered naturally.

The mean Cord Bilirubin (CBB) level was observed to be 2.6±1.06 mg/dL. In a similar study, Bhat JA et al., found that the mean CBB of newborns who developed pathological jaundice was also 2.6±0.6 mg/dL [18]. Likewise, Bhat D and Purohit M from North India, as well as Arulparithi CS et al., from Puducherry, reported comparable values of 2.27±0.76 mg/dL and 2.35±0.20 mg/dL, respectively [Table/Fig-6] [3,6,11-16].

The present study aimed to determine a cut-off value for CBB that could help screen babies with a high probability of developing neonatal jaundice in the future. Using a cord bilirubin level of ≥2 mg/dL, significant hyperbilirubinemia could be predicted with a sensitivity of 80%, specificity of 54%, positive predictive value of 57.97%, and negative predictive value of 75%, as determined by ROC analysis. The cut-off cord bilirubin level identified for this study population was 2.1 mg/dL. Values equal to or above this cut-off can be used

Authors	Place/year of the study	Study type	Sample size	Mean UCB (mg/dL)	Incidence of NNH	UCB cut-off (mg/dL)
Current study	Punjab, India, 2023	Prospective cohort study	113	2.6±1.06	35.4%	2.1
Singh R and Jain H [3]	Rajasthan, India, 2019	Prospective cohort study	240	2.23±0.7	17%	1.79
Jones KDJ et al., [6]	London, UK, 2017	Retrospective cohort study	1411	-	2.7%	2.03
Thumjaa A and Vindhiya K [11]	Chennai, India, 2010-11	Prospective cohort study	129	-	13%	2.65
Bhat D and Purohit M [12]	Punjab, India, 2012	Prospective case control study	100	-	33%	2.5
Kumar TA et al., [13]	Tamil Nadu, India, 2014	Prospective cohort study	135	-	10%	2.3
Eldho HP et al., [14]	Assam, India, 2020-21	Prospective cohort study	120	2.75	21.6%	3.5
Janaki AN and Selvakumar P [15]	Tamil Nadu, India, 2018	Prospective case control study	92	1.995±1.16	24%	1.85
Arulparithi CS et al., [16]	Puducherry, India, 2022	Prospective observational study	50	1.91±0.51	32%	1.95

[Table/Fig-6]: Comparison of Umbilical Cord Bilirubin (UCB) cut-offs from various studies to predict significant Neonatal Hyperbilirubinemia (NNH) [3,6,11-16].

to predict hyperbilirubinemia in neonates with ABO mismatch in Punjab state. Additionally, a positive correlation (p-value <0.05) was found between cord bilirubin and postnatal serum bilirubin in this study.

The negative predictive value of 75% suggests that measuring cord serum bilirubin can help identify neonates who are unlikely to require further intervention and evaluation. Similarly, the largest retrospective study from the UK, involving 1,411 neonates, including 560 ABO-mismatched newborns, found that an arterial UCB cut-off of 2.03 mg/dL (35 µmol/L) could predict neonatal jaundice in ABO-mismatched babies with a post-test probability of 30% [6]. Other similar studies have reported slightly higher UCB cut-offs, ranging from 2.3 mg/dL [13] to 2.65 mg/dL [11]. However, Eldho HP et al., from Assam reported a significantly higher UCB cut-off of 3.5 mg/dL [14]. This discrepancy may be explained by the fact that their study included only caesarean-delivered babies and assessed the UCB cut-off for the risk of jaundice at 24 hours only.

Limitation(s)

The present study has several limitations. The sample size was small, and the results were obtained from a single centre, which limits their generalisability to the entire Indian population. Furthermore, the study only assessed umbilical cord blood specimens from term healthy newborns. Therefore, large-scale multicenter studies involving newborns with varying gestational ages and birth weight categories need to be conducted to establish a UCB cut-off that can predict subsequent hyperbilirubinemia in ABO-mismatched neonates. This would aid in risk stratification and facilitate early discharge.

CONCLUSION(S)

The present study revealed a significant correlation between UCB levels and the development of hyperbilirubinemia in ABO-incompatible term neonates. It was observed that UCB levels ≥2.10 mg/dL can serve as a good predictor for assessing the occurrence of hyperbilirubinemia. Therefore, mothers with O blood group should have their umbilical cord blood group and bilirubin levels assessed at the time of delivery. ABO-incompatible babies with UCB values >2.1 mg/dL should have frequent follow-ups for the timely detection of neonatal jaundice. Conversely, babies with UCB values <2.1 mg/dL may be discharged early from the hospital and require less frequent follow-up.

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Author contributions: AB and JS designed the hypothesis. AB, KB and NS enrolled the subjects and conducted analysis. AB wrote the discussion and conclusion. All authors approved the final manuscript.

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