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Original Research Article

Comparative Evaluation of Neonatal Hyperbilirubinemia Predictors through Umbilical Blood Samples

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Abstract:

Objective: To evaluate and compare the predictive ability of cord blood albumin (CBA), cord blood bilirubin (CBB), and the cord blood bilirubin to cord blood albumin ratio (CBB/CBA) in neonatal hyperbilirubinemia (NNH).

Methods: In this observational prospective analytical study, ninety-six healthy newborns were included after screening for exclusion criteria. Three umbilical cord blood samples were taken with all aseptic precautions for CBA, CBB, and blood group estimation. CBB/CBA was calculated after report collection. A total serum bilirubin sample was sent at 72 hours of life. All the values were statistically analyzed for correlation and comparison in neonates who developed NNH above the recommended phototherapy threshold.

Results: A total of 21 (21.9%) neonates developed NNH above the phototherapy threshold. In the receiver operating characteristic (ROC) curve, the cut-off values obtained for CBA and CBB was 3 g/dL and 2.25 mg/dL, respectively. The sensitivity, specificity, and NPV for CBA were 74.7%, 57.1% and 38.71%, while for CBB they were 95.2%, 56% and 97.67% respectively. The cut-off value for the CBB/CBA ratio obtained was 0.76, with 85.7% sensitivity, 74.7% specificity and a negative predictive value (NPV) of 94.2%.

Conclusions: We recommend CBB/CBA and CBB combined over CBA as it makes the test both highly sensitive and specific. Neonates having a CBB/CBA value of 0.76 or above and a CBB value 2.25 g/dL or above must be followed closely.

Keywords: CBA, CBB, CBB/CBA, NNH, ROC curve.

Introduction

For many decades, neonatal jaundice has been noticed as the most common cause of OPD presentation and NICU readmission as well. A newborn presented with any diagnosis will usually have some degree of jaundice [1]. By affecting about 60-70% of term and 80% of preterm babies, it becomes the number one abnormal physical finding and cause of parental apprehension [2]. The coupling of jaundice pathophysiology with other diseases makes it more lethal and sometimes fatal. Every pediatrician is acquainted with the perilous nature of neonatal jaundice if we take immature neonatal brain into contemplation. A high concentration of unconjugated bilirubin crossing the undeveloped blood-brain barrier is the paramount detrimental reverberation of jaundice. The effects range from seizures and increased tone to apnea and perpetual neurological impairment, such as kernicterus and choreoathetoid cerebral palsy [3]. In this kind of sitch, the role of advanced detection and prediction comes into play. Identifying newborns at risk of developing significant jaundice requiring intervention is key, and the ultimate goal is to limit bilirubin levels below a critical level [4]. But the challenges in developing countries are humongous such as limited resources, socioeconomic factors, pre-lacteal feeds, social rituals and early discharge from the hospital. Therefore, this attractive zone of prediction needs requisite characteristics like being easy to implement, fast, reliable and cost-effective. A simple tool with these characteristics is going to be a game changer. One of them is CBA. The human fetus starts synthesizing albumin at the 7th to 8th week of intrauterine life and binds with bilirubin in a 1:1 ratio [5]. Albumin acts as the chief binding protein, binding with various body components. It is totally produced by the fetal liver,

as albumin does not cross the human placenta. Displacement of bilirubin from albumin leading to a decrease in this equimolar ratio, causes bilirubin toxicity. This fact denotes the role of CBA in predicting the development of significant jaundice. CBB is one of the predominantly studied predictors; its levels have been used to identify neonates who require follow-up.

Measurement of CBB has predictive value in identifying neonates who may develop significant hyperbilirubinemia requiring phototherapy, especially in neonates with blood group incompatibility [6,7]. Another early predictor is the ratio of CBB to CBA. This ratio potentially acts as a proxy for free bilirubin and measures the available or consumed albumin reserve, which guides the clinician in further management plans [4]. The American Academy of Pediatrics (AAP) guidelines from 2004 also recommended the use of the total bilirubin concentration/albumin ratio; however, it has not been widely used by clinicians [8]. In this study, we tried to assess and compare the predictive values of CBA, CBB, and CBB/CBA in NNH.

Methods

This observational prospective analytical study was conducted in the Department of Pediatrics, UIMS Prayagraj, from May 2023 to September 2024 after obtaining due approval from the institutional ethics committee (Ref. No. UIMS/IEC/ECC/05, dated – 10/05/2023).

Sample size calculation (n)-

The Sample Size calculation done by using the formula as

$$n = \frac{Z_{\frac{\alpha}{2}}^2 * P * Q}{l^2}$$

Where

n is the required minimum sample size.

Z = 1.96 at 0.05 level of significance

P = Proportion of Neonatal Jaundice varies from 32.9% to 54.6% (Prevalence). There is no exact prevalence-based study in Uttar Pradesh, India. Therefore, in such cases, unknown prevalence is considered i.e. 50%.

Q = 1-P = 50%

L = 20 % (Relative precision or error)

i.e. 20% of 50% (Prevalence)

Then, n = 96

All healthy term newborns of any birth weight born in the institute between May 2023 and September 2024, either by vaginal delivery or LSCS, were included in the study after screening for exclusion criteria. The exclusion criteria include blood group (Rh & ABO) incompatibility, presence of sepsis, birth asphyxia, meconium aspiration syndrome, jaundice within 24 hours of life, presence of a congenital anomaly, intrauterine infections (TORCH), critically ill, preterm, CBB level > 5 and those whose parents did not give consent. A total of 96 newborns were enrolled in our study after taking complete history regarding their demographic profile, antenatal maternal illness (TORCH-related), perinatal events, and informed written consent from parents. All the details were updated on a pre-designed study pro forma.

Sample Collection: The double-clamped umbilical cord was cut at the placental end after clamping from the neonatal end, with all proper aseptic precautions. Three cord blood samples of 2 ml each were taken by puncturing with a sterile syringe. Two samples were taken in a plain vial and immediately sent for CBA and CBB estimation. One EDTA sample was sent for blood group identification. To prevent hemolysis, squeezing was avoided during the whole procedure [4]. After receiving the reports, CBB/CBA was calculated. The newborns were shifted to the maternal side, and breastfeeding was continued. Now, at 72 hours of life, the babies developed clinically visible jaundice. A 1 ml venous blood sample was sent to the central laboratory for TSB estimation. Bilirubin levels were plotted on the phototherapy thresholds graph given in the revised clinical practice guideline for the management of NNH in newborn infants 35 weeks or more of gestation for infants who have no recognized hyperbilirubinemia neurotoxicity risk factors [9]. TSB levels above the phototherapy thresholds at 72 hours of life, according to gestational age, were shifted to the NICU for phototherapy with breastfeeding.

Statistical analysis- Data were described in terms of range; mean (standard deviation), frequencies (number of cases) and relative frequencies (percentages) as appropriate. For comparison of continuous or ordinal variables, the Mann-Whitney U test was used. Chi square ($\chi 2$) test was performed and exact test was used for comparing categorical data, when the expected frequency is <5. Receiver operator characteristics (ROC) curve was done and Area under curve (AUC) was measured. Criterion value was estimated depending on the specificity and sensitivity. Sensitivity, specificity, positive and negative predictive values and accuracy of each method and its confidence interval (95% CI) were also determined. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21.0 version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

Results

Detail description of baseline characteristics with mean (SD) shown in Table-1.

Table 1: Baseline characteristics of cases

Parameters	
Male n (%)	62 (64.6%)
Female n (%)	34 (35.4%)
Gestational age in weeks (mean (SD))	38.59 (1.23)
Birth weight in Kg (mean (SD))	2.85 (0.36)
CBA (gm/dl) (mean (SD))	3.23 (0.54)
CBB (mg/dl) (mean (SD))	2.15 (0.82)
CBB/CBA (mean (SD))	0.68 (0.29)
TSB at 72 hours (mg/dl) (mean (SD))	20.4 (3.75)

The male-to-female ratio in the study was 1.82 with a minimum of 37 weeks and a maximum of 41 weeks of gestational age.

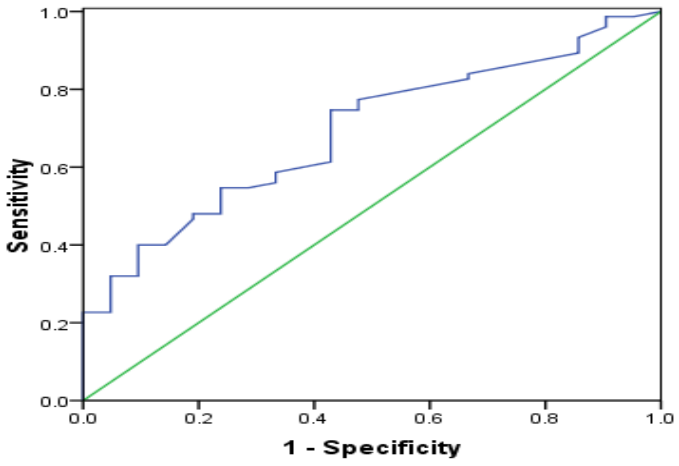
We had a newborn with a birth weight of 1,900 grams which was the lowest among all 96, while a newborn with a birth weight of 3,800 grams was the highest. As we observed the feeding practices, we also found 55 (57.29%) were exclusively breastfed, while 25 (26%) were on mixed feeding such as breast milk and formula feed, and 16 (16.7%) were on formula feed only. While taking maternal history, 3 mothers had a history of previous abortions with unknown causes, and 30 (31.25%) newborns had a history of NNH in their siblings. Out of 96 cases, 21(21.9%) developed jaundice that had a TSB value above the phototherapy threshold according to their gestational age. They were treated with phototherapy. No one developed signs or symptoms of bilirubin encephalopathy or kernicterus and no one required exchange transfusion. We divided neonates in three groups according to values of CBA on the basis of study conducted by Suchanda Sahu et al [10]. First group is CBA <2.9 g/dL, second is CBA 2.9- 3.29 g/dL and third is CBA more than or equal to 3.3 g/dL. On the basis of CBB, all neonates were further classified into 2 groups with cut off value 2.3[11].

Out of total 21 neonates who developed NNH with TSB level above the phototherapy threshold, 11(52.4%) has CBA value <2.9 g/dL, 10(47.6%) has CBA value between 2.9-3.29 g/dL and no one having CBA above 3.3 g/dL developed NNH. In case of CBB groups, out of 21, only 1(4.8%) neonate having CBB value <2.3 mg/dL developed NNH and 20(95.2%) developed NNH with CBB value > 2.3 mg/dL.

Table 2: Representation of the development of significant NNH in the groups divided on the basis of CBA and CBB

		Jaundice After 72 Hrs				Total	Chi-square value	p-value
		No		Yes				
CBA	< 2.9	17	22.7%	11	52.4%	28	7.272	0.026
	2.9-3.29	56	74.7%	10	47.6%	66		
	>3.3	2	2.7%	0	0.0%	2		
Total		75	100.0%	21	100.0%	96		
CBB	< 2.3	42	56.0%	1	4.8%	43	17.418	0.001
	> 2.3	33	44.0%	20	95.2%	53		
Total		75	100.0%	21	100.0%	96		

ROC curves showing sensitivity, specificity, and AUC of CBA, CBB, and CBB/CBA are seen in Figure 1. The full statistical comparison shown in Table 3. On the basis of the cut-off, maximum AUC and specificity are seen in CBB/CBA, while maximum sensitivity and NPV are seen in CBB.



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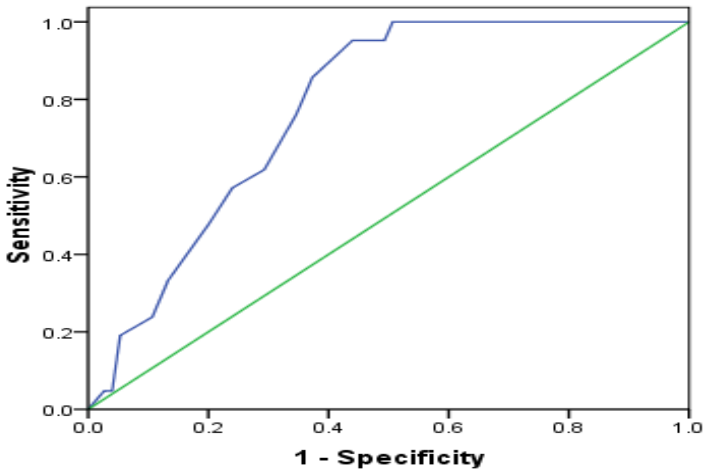

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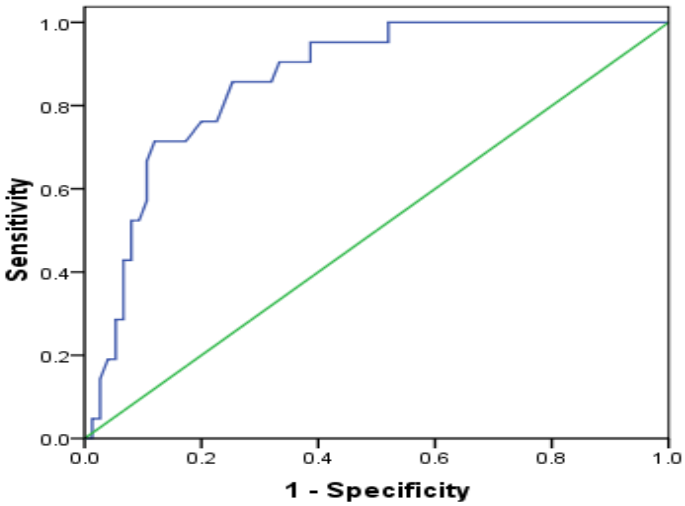

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Figure 1: Showing ROC curves for A) CBA (AUC - 0.690), B) CBB (AUC - 0.777) , C) CBB/CBA (AUC - 0.862)

Table 3: Details statistical comparison of CBA, CBB, and CBB/CBA. A positive correlation is found between all three and the development of NNH (p - value < 0.05)

	Cut-Off Value	Sensitivity	Specificity	NPV	p- Value	95% Ci		STANDARD ERROR
						Lower	Upper	
CBA	3	74.7%	57.1%	38.71%	0.008	0.573	0.807	0.060
CBB	2.25	95.2%	56.0%	97.67%	0.001	0.686	0.869	0.047
CBB/CBA	0.76	85.7%	74.7%	94.92%	0.001	0.785	0.940	0.039

On the basis of the cutoff values obtained in this study, Table 4 shows the results of predictability for significant NNH. Out of 21 who developed significant NNH, a value >0.76 for CBB/CBA predicted 18 (85.7%) cases. CBB at cutoff value > 2.25 mg/dL could predict 20 (95.2%) cases. Both were statistically significant (p-value <0.05).

Table 4: Prediction of NNH by CBA, CBB, and CBB/CBA on the basis of the cut-off value obtained by our study. (All are showing a positive correlation as the p-value for all is < 0.05)

		Jaundice After 72 Hrs				Total	Chi-square value	p-value	
		No		Yes					
CBA (g/dL)	< 3	19	25.3%	12	57.1%	31	7.593	0.006	
	> 3	56	74.7%	9	42.9%	65			
Total		75	100.0%	21	100.0%	96			
CBB (mg/dL)	< 2.25	42	56%	1	4.8%	43	17.419	0.001	
	> 2.25	33	44%	20	95.2%	53			
Total		75	100%	21	100.0%	96			
CBB/CBA	< 0.76	56	74.7%	3	14.3%	59	25.252	0.001	
	> 0.76	19	25.3%	18	85.7%	37			
Total		75	100.0%	21	100.0%	96			

Discussion

Early discharge practices are mostly common in countries with low socioeconomic status and high birth rates. They offer suitable conditions for baby's health like good breastfeeding practices in a secure and safe home environment without maternal anxiety, a strong mother-baby bond, exclusion of further hospital-acquired infections and last but not least, a lesser financial burden on the family. But the question arises in every pediatrician's mind: is this early discharge, with the risk of developing significant NNH, worth taking, given the advantages of early discharge, as it has become the topic of many discussions? If we have defined predictors that can calculate the risk, it will be beneficial for the baby's life and will decrease the economic burden of readmission on the family as well. In our study, we found 21 (21.9%) newborns developed NNH above phototherapy threshold, this result is supported by studies such as 19.86% in Bernaldo et al. [12], 19.84% [4], but contradicted by 10.60% [13], and 56% [14]. We must remember that the development of NNH also depends on some genetic, environmental and ethnic factors. Therefore, the numbers can differ from study to study. We observed a statistically significant (p-value 0.008) CBA cut-off value that of 3 g/dl with sensitivity of 74.7%, specificity of 57.1% and poor NPV 38.71%. This observation is in agreement with the results obtained by Gaurav Aiyappa et al. [15], they found a sensitivity 71.8% and specificity 65.1%. A Similar observation was also made by Trivedi et al. [16], Pahuja et al. [17] and Sahu et al. [18] with different types of inclusion and exclusion criteria, but it is not in agreement with Sahan H et al. [19] as they found CBA not effective for determining the requirement of phototherapy. The cut-off value for CBB in our study was 2.25 mg/dL with sensitivity of 95.2%, specificity of 56% and NPV 97.67%, which is statistically significant for the detection of significant NNH. A.A. Zeitoun et al. [20] conducted a study with 94 newborns and concluded that with a cut-off value of 2.15 mg/dL for CBB in full term babies, it is a predictor of the jaundice severity developed by healthy term babies without complications during the first week of life.

These results are consistent with our results. Results obtained by D. Kardum et al. [21] showed a sensitivity of 76.85% and specificity of 69.58% for CBB with a cut-off value 34 micromoles/l for prediction of NNH. Our results resemble theirs as we also found positive correlation between CBB and NNH, although they found it to be a poor marker for neonatal sepsis. Table 5 shows the statistical performance of CBB/CBA in various

studies and at different cutoffs. They all show a wide range of sensitivity, specificity and NPV but specificity and NPV are on the higher side in each study, which goes in agreement with our results. In a nutshell, we found CBB to be a NNH predictor with the highest sensitivity and NPV, and the positive correlation between CBB/CBA and NNH is in concordance with all these studies, concluding it to be a useful predictor for NNH with the highest specificity and high NPV [4,19,22-25].

Table 5: Comparison of statistical analysis outcomes for CBB/CBA in various recent studies

Study	Cut Off For CBB/CBA	Sensitivity	Specificity	NPV
Ramteke et al., 2018	0.89	95.45%	89.78%	99.58%
Sharma et al., 2020	0.719	97.4%	62.6%	79.61%
Rehna et al., 2021	0.59-0.69	43.5%-65%	64.2%-80.5%	81.9%-85.3%
Venkatraman L et al., 2023	0.86	54.4%	98.4%	NA
Sahan H et al., 2023	0.56	74.2%	61.8%	93.5%
Kosigi A, R. GK., 2023	0.63	91.66%	86.84%	97.05%
Present Study 2024	0.76	85.7%	74.7%	94.92%

Conclusion

Due to certain limitations of this study, such as a limited sample size, the exclusion of preterm infants, exclusion of ABO/Rh incompatibility and septic newborns and limited resources, we need more studies like this on a larger scale. If we find a good NNH predictor in healthy neonates, it will open a way to try them in newborns having risk factors for NNH (like our exclusion criteria). In the end, we conclude that the present study demonstrated that all CBA, CBB, and CBB/CBA are potentially helpful markers as they all showed statistically significant correlations for prediction of significant NNH.

But as our objective was to do comparative evaluation, we found that CBB/CBA has the highest specificity with the highest AUC in ROC curve analysis, while CBB stands out with highest sensitivity and highest NPV, we recommend CBB/CBA and CBB as a combined test over CBA for prediction of NNH. The generalizability of these findings are questionable but the implication of this recommendation in our clinical settings will surely help in early prediction and improving outcome of NNH.

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