Correlation of Cord Blood Bilirubin Values with Neonatal Jaundice Contributors

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ABSTRACT

Background: Neonatal Jaundice (NNJ) is a common disorder worldwide and one of the important contributors to high neonatal morbidity and mortality. Cord Blood Bilirubin samples are easy to collect for estimation of bilirubin levels of new-born at birth. It helps in detecting new-borns who are at a low risk of developing hyperbilirubinemia and minimizes unnecessary prolongation of hospitalization or hospital readmission for hyperbilirubinemia

Material and methods: The study was a prospective observational study conducted in a tertiary care hospital of North India. The sample size was 110 healthy full term new-borns. Cord blood bilirubin (CBB) was estimated at birth. This was followed by clinical examination of all new-borns daily for jaundice and its severity assessed by Kramer's score. Total serum bilirubin(TSB) values was also estimated at 72 hours of age.

Results: New-borns with CBB >2.2 mg/dl had significantly high risk of requirement of phototherapy at 72 hours with adjusted odds ratio of 93.469 (4.117 to 2121.94), 883.760 (25.41 to 30734.86) respectively. This cut-off value had a sensitivity of 87.88%, specificity of

85.71%, positive predictive value of 72.5%, negative predictive value of 94.3% and a diagnostic accuracy of 86.36%.

Conclusion: The study concluded that measurement of umbilical cord blood bilirubin (CBB) level can be used as a screening tool for predicting the development of significant hyperbilirubinemia requiring interventional therapy.

Keywords – Neonatal hyperbilirubinemia, cord blood bilirubin, total serum bilirubin, phototherapy

INTRODUCTION

Neonatal Jaundice (NNJ) is a common disorder worldwide [1,2] and one of the important contributors to high neonatal morbidity and mortality. [3,4] Severe neonatal jaundice leads to brain damage or even death in otherwise healthy new-borns. [5]

Jaundice is observed during 1st week after birth in approximately 60% of term infants and 80% of preterm infants. [6] A small percentage may however require phototherapy or exchange transfusion if the bilirubin levels exceed beyond the normal range. It is important to identify such new-borns who are at risk of developing hyperbilirubinemia, as it can cause acute bilirubin encephalopathy and kernicterus/chronic encephalopathy, which have a high mortality and significant morbidity with long term sequelae. [5,7]

Many times due to family, emotional and social constraints, doctors have to discharge the new-borns early. In such situations, it is difficult to predict which of these new-borns are at risk for developing significant hyperbilirubinemia (Total Serum Bilirubin $\geq 95^{th}$ centile of nomogram). [5] Many investigators have tried to find a simple marker to predict hyperbilirubinemia and its subsequent course in new-borns like cord blood bilirubin

estimation,[8,9] bilirubin estimation during 6 to 24 hours of age,[10-13] predischarge hour specific bilirubin estimation[14] and transcutaneous bilirubin measurement[15-18].

Cord Blood Bilirubin samples are easy to collect for estimation of bilirubin levels of new-born at birth. It helps in detecting new-borns who are at a low risk of developing hyperbilirubinemia and minimizes unnecessary prolongation of hospitalization or hospital readmission for hyperbilirubinemia.[19]

In view of the above facts, the present study is undertaken to correlate the predictive ability of cord blood bilirubin level for subsequent management of hyperbilirubinemia in healthy term new-born.

MATERIALS AND METHOD

The study was a prospective observational study conducted in a tertiary care hospital of North India. The sample size was 110 healthy full-term new-borns (gestational age ≥ 37 weeks to ≤ 42 weeks) weighing ≥ 2500 grams born to healthy mothers were included in the study by systematic random sampling. The neonates excluded were preterm babies < 37 weeks of gestation, babies discharged before 72 hours of life, babies with Rh incompatibility, sick new-borns requiring NICU admission, new-borns with major congenital anomalies and those born to mothers with chronic maternal illness.

After obtaining approval from the Institutional Ethics Committee, written consent was taken from parents of eligible new-borns fulfilling the inclusion criteria and were enrolled in the study. New Ballard Scoring was done to assess the gestational age of the new-borns. Cord blood bilirubin (CBB) was collected from the umbilical cord immediately after the birth and was subjected to centrifugation for 10 mins for separation of serum. CBB assay was

performed in the biochemistry laboratory with VITROS FS 5.1 using azobilirubin/dyphylline method. Serum Bilirubin sample was collected from the peripheral vein via venous puncture at 72 hours of age and was subjected to centrifugation for 10 mins for separation of the serum. Total Serum Bilirubin (TSB) assay was performed in the biochemistry laboratory with VITROS FS 5.1 using azobilirubin/dyphylline method. All new-borns were examined daily for jaundice and its severity assessed visually by Kramer's score [20] and if required, TSB values by laboratory also for the confirmation.

Statistical Analysis

Microsoft EXCEL spreadsheet was used to make the data entry. The final statistical analysis was done with the help of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0.

Categorical variables were expressed in the form of number and percentage. Quantitative data were presented as mean ± standard deviation (SD) and as median with 25th and 75th percentiles (interquartile range). The association of variables which were quantitative in nature were analysed using the independent t test. The association of variables which were qualitative in nature were analysed using the Chi- Square test. Fisher's exact test was used, if any cell had an expected value of less than 5. Receiver operating characteristic (ROC) curve was used to find out the cut off point of Cord bilirubin (mg/dL) for predicting neonatal jaundice. Sensitivity, specificity, positive predictive value and negative predictive value was calculated. Multivariate logistic regression was used to find out independent risk factors of requirement of phototherapy at 72 hours. P value of less than 0.05 was considered statistically significant.

RESULTS

The study was conducted in a tertiary care hospital on 110 healthy term new-borns ≥ 37 weeks to ≤ 42 weeks weighing ≥ 2500 g born to healthy mothers. **Table 1** shows the socio-demographic profiles of the study population.

Table 1: Socio-demographic and laboratory values of study population

Variables		Number (N)	Percentage (%)
Gender	Male	42	38.18
	Female	68	61.82
Gestational Age	$37\text{-}38^{+6}$	65	59.09
	$39\text{-}40^{+6}$	35	31.82
	41 - ≤ 42	10	9.09
Cord bilirubin (mg/dL)	≤ 2.2	70	63.64
	<2.2	40	36.36
Serum bilirubin at 72 hours(mg/dL)	<15	81	73.64
	>=15	29	26.36
Type of delivery	Vaginal delivery	59	53.64
	LSCS	51	46.36
Mother's Blood Group	A+	20	18.19
	B+	39	35.45
	AB+	19	17.27
	O+	32	29.09
Baby's Blood Group	A+	24	21.82
	B+	46	41.82
	AB+	19	17.27
	O+	21	19.09
ABO incompatibility	No	92	83.64
	Yes	18	16.36
Requirement of phototherapy	No	77	70
	Yes	33	30

ROC curves above the diagonal line are considered to have reasonable discriminating ability to predict neonatal jaundice. Discriminatory power of cord bilirubin (mg/dL) (AUC 0.867; 95% CI: 0.789 to 0.925) was excellent. Cord bilirubin (mg/dL) was the significant predictor of neonatal jaundice at cut off point of >2.2 with 86.70% chances of correctly predicting Neonatal jaundice (**Figure 1 and Table 2**).

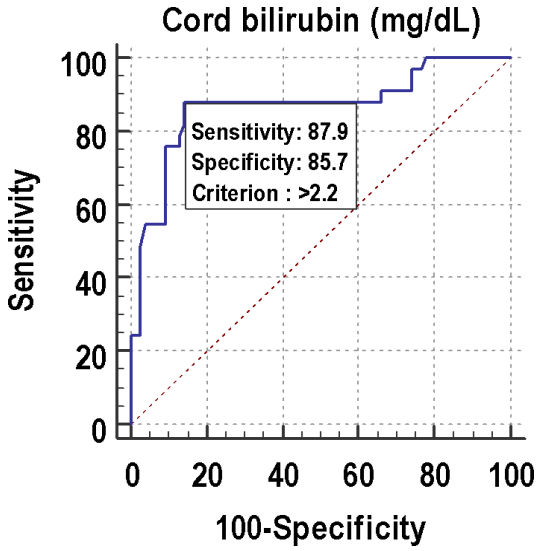

Figure 1: ROC curve of Cord bilirubin (mg/dL) forpredicting neonatal jaundice.

Table 2:-ROC curve of Cord bilirubin (mg/dL) for predicting neonatal jaundice.

Neonatal jaundice	**Cord bilirubin (mg/dL)**
Area under the ROC curve (AUC)	0.867
Standard Error	0.0435
95% Confidence interval	0.789 to 0.925
P value	<0.0001
Cut off	>2.2
Sensitivity(95% CI)	87.88%(71.8 - 96.6%)
Specificity(95% CI)	85.71%(75.9 - 92.6%)
PPV(95% CI)	72.5%(56.1 - 85.4%)
NPV(95% CI)	94.3%(86.0 - 98.4%)
Diagnostic accuracy	86.36%

Association of various categorical and continuous variables with requirement of phototherapy in babies developing pathological jaundice is as shown in **Table 3 and Table 4.**

Table 3 : Association of categorical variables with requirement of phototherapy

Variables		**Phototherapy Not required (n=77)**	**Phototherapy Required (n=33)**	**P value**
Gender of baby	Female	46 (67.65%)	22 (32.35%)	0.493‡
	Male	31 (73.81%)	11 (26.19%)	

Gestational age(weeks)	37 weeks to 38 weeks + 6 days	48 (73.85%)	17 (26.15%)	0.29‡
	39 weeks to 42 weeks	29 (64.44%)	16 (35.56%)	
Type of Delivery	Normal vaginal delivery	42 (71.19%)	17 (28.81%)	0.77‡
	LSCS	35 (68.63%)	16 (31.37%)	
Mother's Blood Group	A+	13 (65%)	7 (35%)	0.55‡
	B+	30 (76.92%)	9 (23.08%)	
	AB+	14 (73.68%)	5 (26.32%)	
	O+	20 (62.50%)	12 (37.50%)	
Baby's Blood Group	A+	13 (54.17%)	11 (45.83%)	0.267‡
	B+	34 (73.91%)	12 (26.09%)	
	AB+	15 (78.95%)	4 (21.05%)	
	O+	15 (71.43%)	6 (28.57%)	
ABO incompatibility	No	69 (75%)	23 (25%)	0.01‡
	Yes	8 (44.44%)	10 (55.56%)	
Cord bilirubin (mg/dL)	<=2.2	66 (94.29%)	4 (5.71%)	<.0001†
	>2.2	11 (27.50%)	29 (72.50%)	
Serum bilirubin at 72 hours(mg/dL)	<15	76 (93.83%)	5 (6.17%)	<.0001†
	>=15	1 (3.45%)	28 (96.55%)	

‡ **Chi square test**

Table 4: Association of various continuous variables with requirement of phototherapy

Variables		**Not required (n=77)**	**Required (n=33)**	Total	**P value**
Gestational age	Mean ± SD	38.59 ± 1.07	38.81 ± 1.19	38.66 ± 1.11	0.343*
	Median (25th-75th percentile)	38.29 (38-39.286)	38.71 (38-39.714)	38.29 (38-39.429)	
	Range	37- 41.14	37- 42	37- 42	
Birth weight	Mean ± SD	2.94 ± 0.29	2.97 ± 0.28	2.95 ± 0.29	0.527*
	Median (25th-75th percentile)	2.86 (2.69-	2.94 (2.78-	2.89 (2.7-	

		3.12)	3.15)	3.128)	
	Range	2.55-3.86	2.54-3.9	2.54-3.9	
Cord bilirubin (mg/dL)	Mean ± SD	1.84 ± 0.45	2.55 ± 0.47	2.05 ± 0.56	<.0001*
	Median (25th-75th percentile)	1.86 (1.51-2.11)	2.66 (2.4-2.79)	2.06 (1.59-2.43)	
	Range	0.78-2.83	1.5-3.34	0.78-3.34	
Serum bilirubin at 72 hours(mg/dL)	Mean ± SD	9.51 ± 2.69	16.01 ± 1.46		<.0001*
	Median (25th- 75th percentile)	9.42 (7.41-11.8)	16.14 (15.66-17)		
	Range	1.83-17.2	11.76-18		

*** Independent t test**

Table 5: Multivariate logistic regression to find out independent risk factors of requirement of phototherapy at 72 hours.

Requirement of phototherapy at72 hours	**Beta coefficient**	**Standard d error**	**P value**	**Odds ratio**	**Odds ratio Lower bound (95%)**	**Odds ratio Upper bound (95%)**
ABO incompatibility	2.852	1.602	0.075	17.314	0.749	400.046
Cord bilirubin (mg/dL)						
≤ 2.2				1.000		
>2.2	4.538	1.593	0.004	93.469	4.117	2121.940
Serum bilirubin at 72 hours(mg/dL)						
<15				1.000		
≥15	6.784	1.811	0.0002	883.760	25.412	30734.868

On performing multivariate regression, cord bilirubin (mg/dL) >2.2 mg/dl, serum bilirubin at 72hours(mg/dL) ≥15 were significant independent risk factors of requirement of phototherapy

at 72 hours after adjusting for confounding factors. Newborns with cord bilirubin (mg/dL) >2.2 mg/dl, and study subjects with serum bilirubin at 72 hours(mg/dL) ≥15 had significantly high risk of requirement of phototherapy at 72 hours with adjusted odds ratio of 93.469 (4.117 to 2121.94), 883.760(25.412 to 30734.868) respectively (**Table 5).**

DISCUSSION

Neonatal jaundice is one of the most common reasons for the readmission of the neonates in the hospital for hyperbilirubinemia after getting discharged from the hospital [21]. The early discharge within 24 - 48 hours of birth is the major concern for readmission. As physiological jaundice peaks around 72 hours of life, the assessment of jaundice is very important at this point. But due to multiple traditional practices, economical constraints, parents take early discharge of their baby and hence further assessment of jaundice is very difficult which further leads to development of subsequent neonatal hyperbilirubinemia.

The study was conducted on 110 healthy new-borns with a mean value of gestational age(weeks) of study subjects was 38.66 weeks ± standard deviation (SD) of 1.1 and mean value of birth weight(kg) of new-borns was 2.95kg ± SD 0.29 kg. The proportion of patients who required phototherapy at 72 hours (55.56%) was significantly higher in patients with ABO incompatibility as compared to patients without ABO incompatibility (25%) (p value=0.01) which was in accordance with the study done by Risemberg et. al. reported that the possibility of developing significant hyperbilirubinemia among new-borns with ABO incompatibility whose CBB was greater than 4 mg/dL was higher and that these babies should be re-evaluated frequently. [22] However, in their study, CBB value was quite higher than in the present study.

Another study by Selma Aktas et. al.[23] demonstrated that the median CBB level of new-borns with ABO incompatibility who required PT was 2.74 mg/dl. This value was quite lower than the results of Risemberg et al[22] but was nearer to the values of the present study. However in contrast, the study done by Haque[24] indicated that CBB was an unreliable marker for predicting hyperbilirubinemia in ABO incompatibility. In the present study there was no significant correlation found between the CBB value and need of phototherapy at 72 hours, for the parameters like gestation, mode of delivery, gender, birth weight.

In the present study, the level of CBB was found to be a predictable marker of neonatal jaundice at 72 hours of life. Neonates developed significant hyperbilirubinemia at 72 hours of life with a mean CBB of 2.05 mg/dl ± SD of 0.56 md/dl and required phototherapy with the mean serum bilirubin of 16.01mg/dl with SD of 1.46 mg/dl which was in accordance with the study conducted by Bhat JA et al[25], where new-borns developed hyperbilirubinemia with the mean CBB of 2.6 mg/dl with standard deviation of 0.8 mg/dl who required phototherapy with the mean serum bilirubin of 16.7 mg/dl with SD of 1.8 mg/dl.

Another conducted by Sehgal, P. et al[26] found that infants developed hyperbilirubinemia with mean CBB of 2.02 mg/dl and mean serum bilirubin at 72 hours. The mean ± SD of TSB at 72 hrs was 10.56 ± 3.18 mg/dl. Similarly, Knudsen[27] in his study demonstrated that a jaundiced new-born presented higher umbilical cord bilirubin levels than a new-born without clinical jaundice. In addition , the number of jaundiced new-borns undergoing phototherapy were significantly higher when the levels of CBB were higher than 2.0 mg/dl. Nahar et al.[28] found that the levels of CBB of 2.5 mg/dl ± 0.5 mg/dl can predict the occurrence of pathological jaundice in neonates.

In the present study, as per the ROC curve, the cut off point of CBB was found to be > 2.2 with a sensitivity of 87.88%, specificity of 85.71%, positive predictive value(PPV) of 72.5%, negative predictive value(NPV) of 94.3%. The strength and association of cord bilirubin > 2.2 mg/dl and requirement of phototherapy at 72 hours was found to be significant with p value <0.001 which was in accordance with the study done by Sehgal, P. et al.[26] The CBB of > 2.02 had sensitivity and specificity of 87.5% and 70.8% respectively with a PPV of 0.39 and NPV of 0.965.

In the study by Bhat et al,[25] CBB value of >3.5 mg/dl had high sensitivity (97.06%), specificity (99.22%), positive predictive value (94.29%), and negative predictive value (99.61%) in predicting development of future pathological jaundice. Another study by Ramamoorthy et al[29] the CBB level of >2 mg/dl had the highest sensitivity (93.3%) and this critical bilirubin level had a very high (98.9%) NPV and fairly low (23.3%) PPV. As per the findings of their study, a critical cut off level of CBB was 2 mg/dl predicted by 90% of the new-borns who developed jaundice.

This study shows that CBB level >2.2 mg/dl can predict with high probability the chances of new-borns developing pathological neonatal hyperbilirubinemia requiring phototherapy. Therefore, these new-borns should be meticulously followed-up for developing neonatal jaundice. CBB cut-off criteria of 2.2 mg/dl showed good specificity and sensitivity, and hence can be used to predict the requirement of phototherapy in a new-born at 72 hours of life.

CONCLUSION

The study concluded that measurement of umbilical cord blood bilirubin (CBB) level can be used as a screening tool for predicting the development of significant hyperbilirubinemia

requiring interventional therapy. The levels of CBB ≥ 2.2 mg/dl in healthy term newborn indicated a 72.50% probability for the need of phototherapy. Measurement of CBB will help paediatricians in making decisions of early discharge of the healthy newborns and thus, minimize unnecessary prolonged hospitalization.

REFERENCES

1. Ogunfowora O, Adefuye P, Fetuga M. What Do Expectant Mothers Know about Neonatal Jaundice. Int Electron J Health Educ. 2006;9:134–40.
2. Melton K, Akinbi HT. Neonatal jaundice. Strategies to reduce bilirubin-induced complications. Postgrad Med. 1999;106(6):167-178. doi:10.3810/pgm.1999.11.775
3. Cashore WJ. Neonatal hyperbilirubinemia. N Y State J Med. 1991;91(11):476-7.
4. Davidson LT, Merritt KK, Weech AA. Hyperbilirubinemia in the newborn. Am J Dis Child. 1941;61(5):958–80.
5. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114(1):297–316.
6. Rajan N, Kommu PPK, Krishnan L, Mani M. Significant hyperbilirubinemia in near-term and term newborns: A case - control Study. J Clin Neonatol. 2017;6(4):220–4.
7. Demott K, Bick D, Norman R, Ritchie G, Turnbull N, Adams C, et al. Clinical Guidelines and Evidence Review For Postnatal Care: Routine PostNatal Care of Recently Delivered Women And Their Babies. National Collaborating Centre for Primary Care and Royal College of General Practitioners. Cited 2009 Feb 12. Pp. 290-296.
8. Bernaldo AJN. Bilirubin dosage in cord blood: could it predict neonatal hyperbilirubinemia Sao Paulo Med J. 2004 May 6; 122(3):99–103.
9. Knupfer M, Pulzer F, Gebauer C, Robel-Tillig E, Vogtmann C. Predictive value of umbilical cord blood bilirubin for postnatal hyperbilirubinemia. Acta Paediatr. 2005 May; 94(5):581–7.
10. Awasthi S, Rehman H. Early prediction of neonatal hyperbilirubinemia. Indian J Pediatr. 1998; 65:131–139.

11. Alpay F, Sarici SU, Tosuncuk HD, Serdar MA, Inanc N, Gokcay E. The Value of First Day Bilirubin Measurement in Predicting the Development of Significant Hyperbilirubinemia in Healthy Term Newborns. Pediatrics. 2000 Aug; 106(2):p.e16.
12. Agarwal R, Deorari AK. Unconjucated Hyperbilirubinemia in Newborn. Indian Pediatr. 2002 Aug 17; 39:30–42.
13. Randev S, Grower N. Predicting neonatal hyperbilirubinemia using first day serum bilirubin levels. Indian J Pediatr. 2010 Feb; 77:147–150.
14. Bhutani VK, Johson L, Sivieri EM. Predictive ability of a predischarge hour specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near term newborns. Pediatrics. 1999 Jan; 103:6–14.
15. Gupta PC, Kumari S, Mullick DN. Icterometer; useful screening tool for neonatal jaundice. Indian Pediatr. 1991 May; 28(5):473–6.
16. Leite MG, GranatoVde A, Facchini FP, Marba ST. Comparison of transcutaneous and plasma bilirubin measurement. J Pediatr (Rio J). 2007 May-Jun; 83(3):283–6.
17. Varvarigou A, Fouzas S, Skylogianni E, Mantagou L, Bougioukou D, Mantagos S. Transcutaneous bilirubin nomogram for prediction of significant neonatal hyperbilirubinemia. Pediatrics. 2009 Oct; 124(4):1052-9.
18. Maisels MJ, Ostrea EM Jr, Touch S, Clune SE, Cepeda E, Kring E et al. Evaluation of a new transcutaneous bilirubinometer. Pediatrics. 2004 Jun; 113(6):1628–35.
19. Maisels MJ, Newman TB. Kernicterus in otherwise healthy, breast-fed term newborns. Pediatrics 1995; 96: 730-733.
20. Kramer LI. Advancement of dermal icterus in the jaundiced newborn. Am J Dis Child. 1969 Sep;118(3):454-8. doi: 10.1001/archpedi.1969.02100040456007. PMID: 5817480.
21. LEE K.S., PERLMAN M. and BALLANTYNE M.: Asso-ciation between duration of neonatal hospital stay and readmission rate. J. Pediatr., 127: 758-766, 1995.
22. Risemberg HM, Mazzi E, MacDonald MG, Peralta M, Heldrich F. Correlation of cord bilirubin levels with hyperbilirubinemia in ABO incompatibility. Arch Dis Child 1977;52(03):219–222.
23. Selma Aktas, MD Caner Dogan, MD Zeynep Hazıroğlu Ökmen, MD Seda Geylani Gulec, MD Is Cord Blood Bilirubin Level a Reliable Predictor for

Developing Significant Hyperbilirubinemia?. Am J Perinatol 2019; 36(03): 317-321.

24. Haque KN. Value of measuring cord blood bilirubin concentration in ABO incompatibility. BMJ 1978;2(6152):1604.
25. Bhat JA, Sheikh SA, Ara R. Correlation of cord blood bilirubin values with neonatal jaundice in healthy newborns: A prospective observational study. Arch Med Health Sci 2019;7:48-52.
26. Sehgal P, Wasim S, Chandar V, Gupta A, Rawat A, Kalra V et al. Cord bilirubin levels as a predictive marker for neonatal hyperbilirubinemia: A prospective study. BMR Medicine. 2017;4(1):1-7.
27. Knudsen A. Prediction and noninvasive assessment of neonatal jaundice in the term healthy newborn infant. ActaPaediatr 1996; 85: 393-7.
28. Nahar Z, Shahidullah MD, Mannan A, Dey SK, Mitra U, Salimuzzaman SM. The value of umbilical cord blood bilirubin measurement in predicting the development of significant hyperbilirubinemia in healthy newborn. Bangladesh J Child Health 2009;33:50‑ 4.
29. Ramamoorthy K, Abilash M.S. Cord blood bilirubin used as an early predictor of hyperbilirubinemia Int J Contemp Pediatr. 2018;5(4):1280-5.