

Original Research Article

CORRELATION OF CORD BLOOD BILIRUBIN AND NEONATAL HYPERBILIRUBINEMIA IN NEWBORNS WITH ABO SETUP IN TERTIARY CARE CENTRE

Kavya M¹, Jayaraj Patil², Venkatesh G², Srinivasa Arer³

¹Senior Resident, Department of Paediatrics, Gadag Institute of Medical Sciences, Gadag, Karnataka, India

²Associate Professor, Department of Paediatrics, Gadag Institute of Medical Sciences, Gadag, Karnataka, India

³Professor And Head, Department of Paediatrics, Gadag Institute of Medical Sciences, Gadag, Karnataka, India

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Corresponding Author:

Dr. Kavya M,
Senior Resident, Department of
Paediatrics, Gadag Institute of Medical
Sciences, Gadag, Karnataka, India
Email: kavyambbs000@gmail.com

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ABSTRACT

Background: ABO incompatibility is the most common cause of hemolytic disease of the newborn with approximately 15% of live births at risk. Attempts have been taken to protect neonates from developing kernicterus by estimating cord blood bilirubin. The objective is to determine the relationship between cord blood bilirubin and occurrence of neonatal hyperbilirubinemia.

Materials and Methods: It is a hospital based prospective study centered in Gadag Institute of Medical Sciences, Mallasamudra, Gadag. The study was conducted during the period of March 2021 to March 2022 in the department of Paediatrics at Gadag institute of Medical science, Mallasamudra, Gadag.

Results: 226 neonates who fulfilled the inclusion and exclusion criteria were enrolled in the study. Out of the 226 babies, 20 babies who could not be followed up were excluded from the study. 8 newborns who got admitted in the NICU for sepsis, neonatal seizures and respiratory distress were excluded from the study. The dropout rate was 12% and the remaining 198 neonates were followed up. Out of 198 patients 83 newborns are seen to have jaundice. In our study around 23.2 % newborns were born by normal vaginal delivery and rest 76.8% other by LSCS. In our study, Primi mothers were 55 % and multiparous mother were 45%, Mean maternal age in our study was 24.89 ± 3.716 . In our study 52 % were male newborns and 48 % were female. No statistical significance was found between sex distribution to development of significant jaundice. In our study 55 % were with birth weight of 2.5-2.9 kgs, 36% with birth weight of 3-3.4 kgs and 9% with birth weight of 3.5 -4 kgs. Birth weight distribution to development of significant jaundice by Pearson chi square test is statistically insignificant. Our study provided cut off values of newborns with cord total bilirubin of $>2\text{mg/dl}$ in ABO blood group incompatibility should be followed up closely to watch for the development of significant hyperbilirubinemia requiring treatment in the form of phototherapy with or without exchange transfusion. 3 out of 83 required exchange transfusion.

Conclusion: Umbilical cord serum total bilirubin of more than or equal 2 mg/dl predicts the development of significant hyperbilirubinemia with a there would be 100% sensitivity and 1 - specificity would be 95.7%.

Keywords: Umbilical cord serum total bilirubin, Hyperbilirubinemia, phototherapy, exchange transfusion.

INTRODUCTION

Neonatal hyperbilirubinemia affecting 60% of term and 80% of preterm babies in the first week of life.^[1] Excess bilirubin production, inability to handle this excess load by the newborn's immature liver

enzymes, poor colonization of the intestines by bacteria, increased brush border beta glucuronidase activity and enhanced enterohepatic circulation together contribute to this increased incidence of hyperbilirubinemia in neonates.

Very high bilirubin levels and kernicterus occur in ABO incompatible healthy term newborns even without significant hemolysis and positive direct agglutination test.^[2] Acute bilirubin encephalopathy (ABE) may ensue and evolve into kernicterus (chronic bilirubin encephalopathy), a permanent disabling neurologic condition classically characterized by the movement disorders of dystonia and/or choreoathetosis, hearing loss caused by auditory neuropathy spectrum disorders, and oculomotor pareses.^[3]

Early postnatal discharge of healthy term newborns within 48 hours of life has become common nowadays. This reduction in hospital stay allows the family to return to their daily routine at the earliest and reduces the economic burden on them in a developing country like India. Carty EM and Bradley CF in their study, A randomized, controlled evaluation of early postpartum discharge, found that mothers who were discharged from the hospital earlier were significantly more satisfied with the care and had higher exclusive breast feeding rates than the late discharge group. While those mothers in the late discharge group scored higher on measures of depression and lower in confidence.^[4]

Total serum bilirubin (TSB) is the measure of albumin-bound bilirubin, whereas the small circulating fraction not bound to albumin or other serum proteins is indexed by the unbound or —free Bilirubin (Bf) level. There is a keen interest in circulating Bf, its measurement, and its ability to predict bilirubin-induced neurologic injury. Indeed, Bf is the vehicle of bilirubin's biologic effects in the brain. However, bilirubin-induced neurotoxicity depends on a complex interaction between the level and duration of central nervous system (CNS) Bf exposure and the innate cellular characteristics of the developing CNS that may predispose or protect against bilirubin-induced neuronal injury, and the clinical laboratory measurement of circulating Bf is not generally available.^[3] As a result, clinicians must rely on the TSB and the bilirubin/albumin (B/A) ratio, an imperfect surrogate of circulating Bf, to index the risk for ABE and drive treatment. Albumin binds in equimolar concentrations with bilirubin and free bilirubin levels in serum increase at times of low serum albumin concentration. Similar correlations between umbilical cord serum bilirubin and neonatal hyperbilirubinemia also have been discussed for many years now.

Since then, many researchers studied the correlation between the umbilical cord blood bilirubin and albumin in predicting significant neonatal hyperbilirubinemia.

However, no study has established a single cut off value for umbilical cord serum bilirubin especially in ABO incompatibility to allow us to predict at birth those babies who will develop significant hyperbilirubinemia to require therapeutic intervention.

Thus, the present study was conducted to evaluate the predictive ability of the umbilical cord blood bilirubin and albumin for significant neonatal hyperbilirubinemia in ABO incompatibility.

MATERIALS AND METHODS

This Prospective cross sectional study was conducted in Gadag Institute of Medical Sciences, Mallasamudra, Gadag. The study will be conducted during the period of March 2021 to March 2022 in the department of Paediatrics at Gadag institute of Medical science, Mallasamudra, Gadag.

Sample Size: 198

Sampling method: Purposive sampling

Inclusion Criteria

- Healthy Term Neonates with A or B blood group born to healthy mother with O positive blood group
- Born by normal delivery or cesarean section
- Birth weight 2.5kg to 4kg
- Apgar score of 7 or more at 1 min

Exclusion Criteria

- Rhesus blood factor incompatibility
- Significant illness requiring NICU admission
- Major congenital malformation
- Delivered by instrumental delivery
- h/o meconium stained liquor or at risk of sepsis [premature rupture of membranes more 12 hours]

Statistical Method: The data was entered and analysed using the latest SPSS 24th edition, the results were calculated using Pearson correlation coefficient calculating ROC, Sensitivity, Specificity of the test and Regression analysis. Statistical association by chi square test

RESULTS

In our study, out of 198 newborns 94 were females and 104 were males. Out of 198 neonates, 83 neonates are seen to have jaundice.

In our study 47% that is 49 out of 104 of male developed jaundice and 36% that is 34 out of 94 of female developed jaundice. Total of 41 % developed jaundice that is 83 in total developed jaundice. In our study around 46 neonates were born by normal vaginal delivery and rest other by LSCS.

Table 1: Comparison of mode of Delivery and development of significant jaundice.

Mode of Delivery	Count of Jaundiced newborns	Percent
LSCS	69	83%
NVD	14	17%
Total	83	100.0%

In our study out of 152 neonates born by LSCS, 69 neonates developed significant jaundice and 14 out of 46 neonates born by NVD developed significant jaundice.

In our study 89 babies were born to multiparous mother and rest to primigravida.

Table 2: Distribution of parity in comparison with development of significant jaundice.

Parity	Count of newborns developed significant jaundice	Percent
MULTI	31	38%
PRIMI	52	62%
Total	83	100.0%

In our study out of 89 babies which were born to multiparous mother, 31 babies developed significant

jaundice. 52 out of 109 babies which were born in primigravida mothers, developed jaundice.

Table 3: Distribution of Birth Weight

Birth	Count	Percent
2.5-2.9 kg	110	55%
3.0-3.4 kg	72	36%
3.5-4.0kg	16	9%
Total	198	100.0%

Around 110 neonates were between 2.5-2.9 kg of weight which is highest. 72 neonates were born with weight between 3.0-3.4 kgs and 16 with 3.5-4.0 kgs. Among birth weight of 2.5 – 2.9 kgs, out of 110 newborns, 46 developed jaundice. With birth weight of 3.0-3.4 kgs 27 out of 72 newborns developed jaundice. Among birth weight of 3.5-4.0 kgs 10 out of 16 newborns developed jaundice. Majority of the patients developed jaundice at 49-72 hrs of life that is 59 patients. Least number of patients that is 2 were seen to develop jaundice after 72 hours.

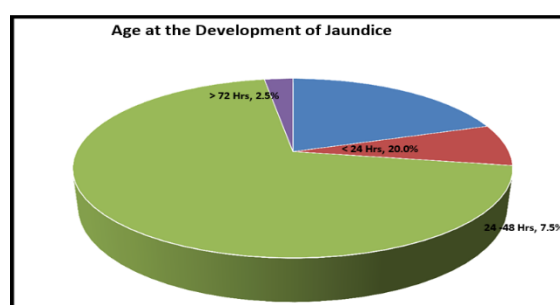


Figure 1: Age at development of jaundice is depicted in the pie diagram.

In our study, total number of neonates with A positive blood group is 102 that constitute 51% and with B blood group is 96 that constitute 48%.

Table 4: Baby Blood and Jaundice

BabyBlood Group	Count			%		
	Jaundice		Total	Jaundice		Total
	Yes	No		Yes	No	
A Positive	44	58	102	43%	57%	51.5%
B Positive	39	57	96	40%	60%	48.5%
Total	83	115	198	41%	59%	100.0%
Pearson Chi-Square		.257a	df	1	Sig	0.612
Not Significant						

Among neonates with blood group A 44 out of 102 developed jaundice that constitute to 43% and among neonates with blood group B 39 out of 57 developed

Jaundice. By Pearson chi square test is statistically insignificant.

Table 5: Cord serum bilirubin total

C.S.B Total	Count	Percent
0-0.9	13	6.6%
1-1.4	44	22.2%
1.5-1.9	59	29.8%
2.0-2.4	46	23.2%
2.5-2.9	15	7.6%
3-3.4	9	4.5%
3.5-3.9	3	1.5%
4.0-4.5	7	3.5%
> 4.5	2	1.0%
Total	198	100.0%

Highest number of neonates were seen to have C. S. bilirubin total of 1.5 to 1.9 mg/dl that is 59 neonates and least number of neonates that is 2 seen to have C. S. bilirubin total of >4.5.

Table 6 – Cord serum bilirubin and Jaundice

C.S.B	Count			%		
	Jaundice		Total	Jaundice		Total
	Yes	No		Yes	No	
0-0.9	1	12	13	7%	93%	6.6%
1-1.4	1	43	44	0.2%	99.8%	22.2%
1.5-1.9	13	46	59	22%	78%	29.8%
2.0-2.4	33	13	46	71%	29%	23.2%
2.5-2.9	14	1	15	93%	7%	7.6%
3-3.4	9	0	9	100%	0%	4.5%
3.5-3.9	3	0	3	100%	0%	1.5%
4.0-4.5	7	0	7	100%	0%	3.5%
> 4.5	2	0	2	100%	0%	1.0%
Total	83	115	198	41.4%	58.6%	100.0%
Pearson Chi-Square		104.407a	df	8	Sig	0.000
Significant						

C.S.Bilirubin -2.0-2.4 mg/dl, 33 out of 46 neonates developed significant jaundice and C.S.Bilirubin of 2.5-2.9 mg/dl, 14 out of 15 neonates developed significant jaundice. Similarly C.S.Bilirubin of 3-3.4mg/dl, 3.5-3.9mg/dl, 4.0-4.5mg/dl and >4.5mg/dl all neonates developed jaundice. C.S.Bilirubin with range of 0-0.9 mg/dl, 1-1.4 mg/dl, 1.5-1.9 mg/dl developed less jaundice like, 1 out of 13, 1 out of 44

and 13 out of 59 neonates developed significant jaundice.

Around 83 neonates were given phototherapy among which maximum number of phototherapy was given for a period of 1 day that is 41 and second maximum number of days for which phototherapy given was for 2 days that is 30 neonates. Least of neonates that is 2 were given phototherapy for 4 days.

Table 7: Exchange Transfusion

Exchange Transfusion	Count	Percent
Yes	3	3.75%
No	80	96.25%
Total	83	100.0%

Out of 83 babies for which exchange transfusion needed, only 3 were given exchange transfusion. ROC Curve between Cord Serum Bilirubin Total and Jaundice

The sensitivity vs. specificity values is plotted in the ROC. curve as the cutoff point moves from 0 to 1. In the study, there were 198 patients, of which 83 of them had jaundice, and the remaining 115 did not.

Table 8: ROC Curve between Cord serum bilirubin total and jaundice

Jaundice	Valid N (listwise)
Positive	83
Negative	115
Larger values of the test result variable(s) indicate stronger evidence for an actual positive state.	
a. The actual positive state is Yes.	

A model with high sensitivity and specificity will have a ROC curve that touches the upper left corner of the plot. A curve nearly perpendicular to the diagonal line at a 45-degree angle indicates a model with low sensitivity and specificity. From the study of 198 neonates, it is clear that the model does a decent job of forecasting the jaundice CUTOFF.

The area under the curve indicates the model's ability to distinguish between a neonate with and without jaundice. The model performs better at accurately classifying outcomes when the AUC is larger. However, the AUC value in the study is 0.907 when the significance value is 0.000, which is comparatively higher in predicting the jaundice CUTOFF when C.S.Bilirubin total is in place.

Table 9: Area Under the R.O.C. Curve between Cord serum bilirubin total and jaundice

Area Under the Curve				
Test Result Variable(s):				
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.907	0.022	0.000	0.863	0.950
The test result variable(s): C.S.B TOTAL has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

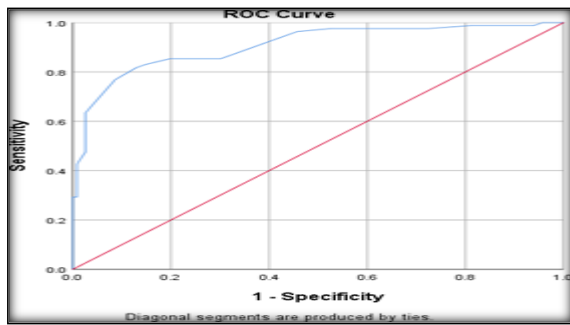


Figure 2: Graph depicting R.O.C. Curve between Cord serum bilirubin total and jaundice

In the ROC curve's sensitivity and specificity for various cutoff, positions are shown in the final table. Here the threshold of 0.7000 has been taken; then in that case, it can predict that neonate with a C.S.Bilirubin Total value above the given cutoff will be considered to have jaundice else, the neonates does not have jaundice. By using the given cutoff, there would be 100% sensitivity and 1 - specificity would be 95.7%.

Table No 10. Coordinates of the Curve between Cord serum bilirubin total and jaundice

Coordinates of the Curve		
Test Result Variable(s):		
Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
-0.6000	1.000	1.000
0.4500	1.000	0.991
0.5500	1.000	0.974
0.7000	1.000	0.957
The test result variable(s): C.S.Bilirubin TOTAL has at least one tie between the positive actual state group and the negative actual state group.		
a. The smallest cutoff value is the minimum observed test value minus 1, and the largest is the maximum value minus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.		

There is a positive correlation of 62.9% between C.S.Bilirubin Total and Jaundice.

Table 11: Correlation between Cord serum bilirubin total and jaundice

		Jaundice	Result
C.S.B total	Pearson Correlation	.629**	Significant
	Sig.	0.000	
	N	198	
**. Correlation is significant at the 0.01 level (2-tailed).			

Significance Level between 72 hrs serum bilirubin indirect and Jaundice.

There is a significant difference between the 72 hrs Serum Bilirubin and Jaundice as per 83 jaundice and 115 non-jaundice neonates

Table 12: Independence T-Test for 72 hrs serum bilirubin indirect and jaundice

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.	Mean Difference	Std. Error Difference	95% CI of the Difference	
72	Equal variances assumed	28.258	0.000	11.87	196	0.000	4.636	0.391	3.866	5.407
H.R.S. S.B.I	Equal variances not assumed			10.69	109	0.000	4.636	0.434		5.496

DISCUSSION

226 neonates who fulfilled the inclusion and exclusion criteria were enrolled in the study. Out of the 226 neonates, 20 neonates who could not be followed up were excluded from the study. 8 neonates who got admitted in the NICU for sepsis, neonatal seizures and respiratory distress were excluded from the study. The dropout rate was 12 % and the remaining 198 neonates were followed up. Out of 198 neonates, 83 neonates are seen to have jaundice.

In our study around 46 neonates were born by normal vaginal delivery and rest other by LSCS. As because early discharge in normal vaginal delivery many cases are missed out.

In our study 89 neonates were born to multiparous mother and rest to primigravida that is Primi mothers were 55 % and multiparous mother were 45%, Mean maternal age in our study was 24.89 ± 3.716 .

Around 110 out of 198 were with birth weight of 2.5-2.9 kgs which constitute 55 %. neonates with birth weight of 3-3.4 kgs constituted with 36 % and 3.5-4 kgs constituted with 9%. By Pearson chi square test

it is statistically insignificant in relation to birth weight and development of significant hyperbilirubinemia. Mean birth weight in our study was 2.932 ± 0.333 kgs.

In our study, out of 198 neonates 94 were females and 104 were males. In our study 47 % that is 49 out of 104 of male developed jaundice and 36% that is 34 out of 94 of female developed significant jaundice. Total of 41 % developed significant jaundice. Significant neonatal jaundice in Sex distribution in our study is statistically insignificant.

In our study, total number of neonates with A positive blood group are 102 that constitute 51 % and with B blood group is 96 that constitute 48%. Among neonates with blood group A 43% that is 44 out of 102 developed jaundice. Among neonates with blood group B, 38 out of 96 developed significant jaundice which constitute for 40%. By Pearson chi square test it is statistically insignificant.

Majority of the neonates developed jaundice at 49-72 hours of life is 59 out of 83 neonates that constitute about 70 %. Least number of neonates 2 out of 83 that constitute of 2.5% are seen to develop jaundice after 72 hours. Less than 24 hours 20% neonates developed significant jaundice that is 16 out of 83 neonates. In 24-48 hours about 7.5 % that is 6 out of 83 neonates developed significant jaundice. Mean age of development of significant jaundice in our study is 60.57 ± 20.77 hrs.

In our study C.S.Bilirubin of > 4.5 mg/dl was seen in only 2 neonates (which was the lowest number) which constitute for 1% and maximum number of neonates were seen with Total serum bilirubin of 1.5-1.9 mg/dl which constitute for 29.8%.

In our study with neonates with C.S.Bilirubin of > 2.0 mg/dl developed significant jaundice. CSB -2.0-2.4 mg/dl, 32 out of 46 neonates developed significant jaundice and C.S.Bilirubin of 2.5-2.9 mg/dl, 14 out of 15 neonates developed significant jaundice. Similarly C.S.Bilirubin of 3-3.4 mg/dl, 3.5-3.9 mg/dl, 4.0-4.5 mg/dl and > 4.5 mg/dl all neonates developed jaundice. C.S. Bilirubin with range of 0-0.9 mg/dl, 1-1.4 mg/dl, 1.5-1.9 mg/dl developed less jaundice like, 1 out of 13, 1 out of 44 and 13 out of 59 neonates developed significant jaundice. Mean C.S. Bilirubin is 1.912 ± 0.883 .

In Gouri Rajput et al conducted a study in 2018, which showed the neonates with C.S. Bilirubin of > 2.0 mg /dl had significant jaundice with sensitivity of 86% specificity of 40%, positive predictive value of 29% and negative predictive value of 91%, which was very similar to our study.^[5]

In Janaki et al study conducted in 2017, the cut-off value for umbilical cord serum total bilirubin for development of significant hyperbilirubinemia was 1.85 mg/dl. This value predicts the development of significant hyperbilirubinemia with a sensitivity of 70.6%, specificity of 82.7% and an accuracy of 76.7%, in this study cutoff was less compared to my study.^[6]

Ashish Pradhan et al study conducted in 2017, a cord bilirubin level ≥ 2.50 mg/dl has sensitivity of 84.1%,

specificity of 88.5%, positive predictive value of 98% and negative predictive value of 45.1% for predicting the risk of developing pathological jaundice. This study also correlate with our study.^[7]

In study of Tridevi et al conducted in 2013, cord serum unconjugated bilirubin level of ≥ 2.0 mg/dl and total cord serum bilirubin level of ≥ 2.5 mg/dl appeared as high risk indicator towards predicting neonatal hyperbilirubinemia, this study correlate well with my study.^[8]

In study of Zakianahar et al conducted in 2009, cord blood bilirubin > 2.5 mg/dl had the high sensitivity (77%) and specificity (98.6%) to predict the newborn who would develop significant hyperbilirubinemia. At this level the negative predictive value was 96% and positive predictive value 91%. this study also support to my study statistically.^[9]

By ROC analysis, the cut-off point for umbilical cord serum total bilirubin for development of significant hyperbilirubinemia for the study population was 2 mg/dl. This value predicts the development of significant hyperbilirubinemia, Here the threshold of 0.7000 has been taken; then in that case, it can predict that neonates with a C.S.Bilirubin Total value above the given cutoff will be considered to have jaundice else, the neonates does not have jaundice. By using the given cutoff, there would be 100% sensitivity and 1 - specificity would be 95.7%. The model performs better at accurately classifying outcomes when the AUC is larger. However, the AUC value in the study is 0.907 when the significance value is 0.000, which is comparatively higher in predicting the jaundice CUTOFF when C.S.Bilirubin Total is in place.

Around 83 neonates were given phototherapy among which maximum number of phototherapy was given for a period of 1 day that is 41 neonates with significant hyperbilirubinemia and second maximum number of days for which phototherapy given was for 2 days that is 27 neonates with significant jaundice. Least of 2 neonates with significant jaundice were given phototherapy for 4 days.

3 out of 83 neonates developed significant hyperbilirubinemia requiring exchange transfusion in my study.

CONCLUSION

In umbilical cord blood, the mean serum total bilirubin in babies with blood group A+ve or B+ve born to O+ve mothers was 1.912 ± 0.883 mg/dl respectively. With maternal mean age being 24.89 ± 3.716 and mean birth weight in study is 2.932 ± 0.333 . 72 hours serum total bilirubin mean value being 11.60 ± 3.539 . Mean age of development of significant jaundice 60.57 ± 20.77 hours.

The umbilical cord total bilirubin correlates well with the development of significant neonatal hyperbilirubinemia requiring treatment in the form of phototherapy with or without exchange transfusion. Umbilical cord serum total bilirubin of more than or equal 2 mg/dl predicts the development of significant

hyperbilirubinemia with a there would be 100% sensitivity and specificity would be 95.7%.

We recommend routine measurement of serum bilirubin levels in umbilical cord blood at birth. Neonates with total bilirubin of >2mg/dl and albumin<3.5 g/dl in ABO blood group incompatibility should be followed up closely to watch for the development of significant hyperbilirubinemia requiring treatment in the form of phototherapy with or without exchange transfusion while those neonates with cord total bilirubin <2 mg/dl and albumin>3.5 g/dl can be safely discharged early.

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