

# Role of Cord Blood Serum Bilirubin and 24 Hour Serum Bilirubin in Predicting the Neonatal Hyperbilirubinaemia on the 5<sup>th</sup> day of Life

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## ABSTRACT

### BACKGROUND

Hyperbilirubinemia is one of the most common clinical signs encountered in newborns and it is a universal problem, which creates anxiety and apprehension among the parents and paediatricians as well. Jaundice is a cause of concern not only due to its staining character but also because of potential brain damage caused by it. It is one of the preventable causes of deafness and mental retardation. Though in most cases it is a benign problem nevertheless untreated, severe unconjugated hyperbilirubinaemia is potentially neurotoxic and conjugated hyperbilirubinemia is a harbinger of underlying serious illness.

### METHODS

The study was conducted at King George Hospital Visakhapatnam in the Department of Paediatrics. The study protocol was approved by the ethical committee for research studies of Andhra Medical College, Visakhapatnam. The study population consisted of term new-borns born in the department of obstetrics and gynaecology, King George Hospital, Visakhapatnam. Study design was cross-sectional study. Study was conducted from August 2017 to October 2018.

### RESULTS

The study consisted of a total of 200 cases (N=200). Males (55.5%) were more in number than females (44.5%). Multigravida women (55.5%) were more than primigravida women (44.5%). Gestational hypertension is present in 33% of the females. Pre-mature rupture of membranes is present in 53% of the mothers. Intrapartum drug is administered in 33.5% of the mothers. Mode of delivery was vaginal delivery in 62% women, caesarean section in 35% women and forceps delivery in 3% women. Sibling history is present in 11.5% patients. Descriptive statistics of all the babies is as follows- mean birth weight was 2.85 Kg, mean APGAR at 1 min was 7.78, mean APGAR at 5 min was 9.82, mean APGAR at 10 min was 10.0, mean cord blood bilirubin was 1.40, mean 24 hr serum bilirubin was 4.85, mean 5<sup>th</sup> day serum bilirubin was 13.38 and mean HB was 16.47. Descriptive statistics of all the jaundiced babies is as follows- mean birth weight was 2.97 Kg, mean APGAR at 1 min was 7.79, mean APGAR at 5 min was 9.88, mean APGAR at 10 min was 10.0, mean cord blood bilirubin was 2.32, mean 24 hr serum bilirubin was 6.39, mean 5<sup>th</sup> day serum bilirubin was 18.38 and mean HB was 16.54.

### CONCLUSIONS

There was a statistically significant correlation between 24 hr serum bilirubin and jaundiced patients and also between 5<sup>th</sup> day serum bilirubin and jaundiced patients. There was a statistically significant correlation between cord bilirubin and 24 hr serum bilirubin. Thus, cord blood bilirubin and 24 hr serum bilirubin have role in early prediction of developing significant jaundice in the first week of life.

### KEYWORDS

Jaundice C23.550.429.500 Newborn M01.060.703.520 Kernicterus C10.228.140.163.480

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## BACKGROUND

Neonatal Jaundice is evident in two thirds of entirely healthy term newborns and in a greater proportion of preterms (80%) in the first week of life. 6.1% of term newborns, who are otherwise normal have a maximal serum bilirubin over 12.9 mg/dL<sup>2</sup>. This is a reflection of immature excretory pathway of liver for bilirubin at a time of heightened production. The resultant jaundice is referred to as physiological jaundice. However non physiological or pathological Hyperbilirubinemia is known to occur in 5-10% of healthy term newborns and is the most common reason for readmission of neonates in the first week of life in the current era of early postnatal discharge from the hospital. As there is no cut off value of bilirubin level that can cause bilirubin encephalopathy, neonatal jaundice has become a serious cause of concern for both parents and paediatricians as well. Thus every jaundiced baby necessitates attention at the earliest to look for the features of pathological jaundice. Kernicterus in such Newborns is preventable, provided excessive hyperbilirubinemia for age is promptly identified and appropriately treated. In fact exaggerated physiological jaundice is the most commonly reported cause for readmission during the early neonatal period, and 0.36% of healthy term newborns discharged after 72 hours of life.<sup>1</sup> With the intent to facilitate such identification and treatment, universal screening for severity of bilirubinaemia before hospital discharge may predict, that extraordinary segment of the neonatal population which is at risk for excessive hyperbilirubinemia during the first week after birth.

### Aim

To estimate and correlate cord blood serum bilirubin and serum bilirubin levels of the neonate at 24 hours life with serum bilirubin levels of the neonate on 5<sup>th</sup> day of life.

### Objectives

1. To determine the predictive value of cord blood and 24<sup>th</sup> hour serum bilirubin levels in identifying newborn babies at risk of developing significant hyperbilirubinemia.
2. To establish the cut off values of the cord bilirubin and 24<sup>th</sup> hour serum bilirubin levels to identify such high-risk neonates.

## METHODS

The study was conducted at King George Hospital Visakhapatnam in the Department of Paediatrics. The study protocol was approved by ethical committee for research studies of Andhra Medical College Visakhapatnam. The study Population consisted of Term newborns born at department of obstetrics and gynaecology King George Hospital Visakhapatnam. Study design was Cross – sectional study. Study was conducted from August 2017 to October 2018.

Sample size was 200 (N= 200). The sample size was determined according to the formula:

$$n = \frac{za^2 * pq}{d^2}$$

Where, n is the required sample size, Z a is the standard normal deviate, which is equal to 1.96 at 95% confidence interval. p is the prevalence in the population of the factor under study. q = 100 - p.d. = Absolute precision p = 3.5 (Ahire N et al). q = 96.5, d = 1.768 is taken as absolute precision. Substituting the values in the equation.

$$n = \frac{za^2 * pq}{d^2}$$

$$= \frac{(1.96)^2 * 3.5 * 96.5}{(2.6)^2}$$

$$= \frac{1297.50}{6.76}$$

$$= 191.937 = \text{rounded to } 200$$

Data was entered into Microsoft Excel sheet and analysed using SPSS 21 version software. Categorical data will be represented in the form of frequencies and proportions. Chi-squares used for qualitative data analysis. Continuous data was represented as mean and standard deviation. Independent t test was used to analyse quantitative data. P Value < 0.05 is considered as statistically significant. Collection of Data started with estimation of gestational age of each baby with the aid of LMP. For feasibility only those babies, whose families were residents of Visakhapatnam, who could be followed up till the end of the study on day 5 were taken into the study.

Soon after delivery before clamping the cord, Cord blood is collected and Serum bilirubin is estimated. Blood grouping, typing and Hb% were also done. Venous blood sample of Baby was taken at 24 hrs ± 6 hrs of postnatal age for estimating total serum bilirubin levels. Bilirubin levels were analysed by spectrophotometry method.

This method is based on the fact that bilirubin absorbs light approximately at 454 nm and haemoglobin absorbs light equally at 454 nm and 540 nm as well. The effect of haemolysis was eliminated by subtracting the 540 nm absorbance from 454 nm and thus only bilirubin absorbance is measured. This assay is suitable for neonates less than 2 to 3 weeks only. Blood grouping & Rh typing of mother & baby, serum bilirubin total & direct were done whenever necessary. Those babies with Jaundice on day 1 and within 5 days of postnatal age were examined and managed appropriately. Rest of the babies was followed till day 5 for the evidence of neonatal jaundice. Day 5 serum bilirubin was estimated for those icteric babies, who had yellowish discoloration extending below the knees. Babies with icterus up to the palms and soles and with total serum bilirubin levels >15 mg/dL were kept under phototherapy and exchange transfusion was done as per the unit guidelines. Babies with significant jaundice on day 5 were investigated further.

**Inclusion Criteria**

- Term newborns, born during the study period at King George Hospital, Visakhapatnam, Andhra Pradesh.
- Parents who were residents of Visakhapatnam town.
- Newborns whose parents have given informed written consent for participating in the study.

**Exclusion Criteria**

- Extremely low birth weight babies.
- Neonates with major congenital malformations.
- Neonates who have not been brought for follow up blood sample or whose parents refused to continue in the study.

**RESULTS**

In the present study, males (55.5%) constituted more in number than females (44.5%). multigravida women (55.5%) were more than primigravida women (44.5%). Gestational hypertension was present in 33% of the females. Pre-mature rupture of membranes was present in 53% of the mothers. In the present study, intra partum drug oxytocin was administered in 33.5% of the mothers. We also found that those mother administered oxytocin, high levels of serum bilirubin were observed in their babies. But it was not statistically significant.

In the present study, mode of delivery was vaginal delivery in 62% women, caesarean section in 35% women and forceps delivery in 3% women. Descriptive statistics of all the babies is as follows- Mean birth weight was 2.85 Kg, mean APGAR at 1 min was 7.78, mean APGAR at 5 min was 9.82, mean APGAR at 10 min was 10.0, Mean Cord Bilirubin was 1.409, Mean 24 hr serum bilirubin was 4.85, mean 5<sup>th</sup> day serum bilirubin was 13.38 and mean HB was 16.47 gram%.

	Jaundice	N	Mean	S.D.	t	P
B Wt kg	Yes	33	2.97	0.54	1.59	0.11
	No	167	2.83	0.41		
APGAR at 1 min	Yes	33	7.78	0.48	0.14	0.88
	No	167	7.77	0.57		
APGAR at 5 min.	Yes	33	9.87	0.41	0.68	0.49
	No	167	9.80	0.55		
Cord Bilirubin	Yes	33	2.32	0.09	9.60	0.001
	No	167	1.22	0.04		
24hr Sr Bilirubin	Yes	33	6.39	0.88	11.38	0.001
	No	167	4.54	0.84		
5th day Sr Bilirubin	Yes	33	18.38	1.31	25.06	0.001
	No	167	12.39	1.24		

**Table 1. Comparing Means of the Two Independent Groups by Student t Test**

Descriptive statistics of all the jaundiced babies was as follows- Mean birth weight was 2.97 kg, mean APGAR at 1 min was 7.79, mean APGAR at 5 min was 9.88, mean APGAR at 10 min was 10.0, Mean Cord Bilirubin was 2.32, Mean 24 hr serum bilirubin was 6.39, mean 5<sup>th</sup> day serum bilirubin was 18.38 and mean HB was 16.54 Gram%.

In the present study, among jaundiced patients- males (57.6%) predominate females (42.4%). Among jaundiced patients- multigravida women (57.6%) were more than primigravida women (42.4%). Among jaundiced patients- gestational hypertension was present in 42.4% of mothers. In the present study, among jaundiced patients- intra partum drug oxytocin was administered in 39.4% of the mothers. There was a statistically significant correlation between 24 hr serum bilirubin and jaundiced patients and also between 5<sup>th</sup> day serum bilirubin and jaundiced patients (table-1).

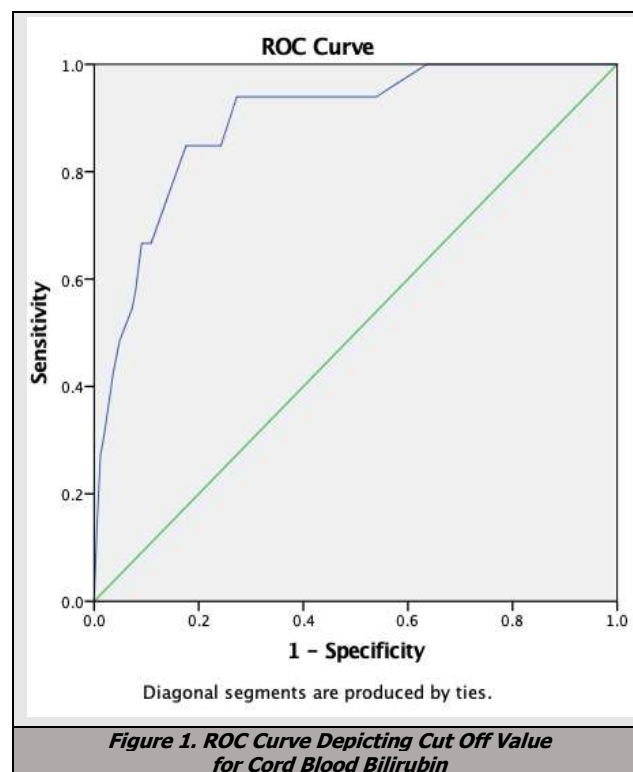
There was a statistically significant positive correlation between Cord Bilirubin and 24 hr serum bilirubin and also between Cord Bilirubin and 5<sup>th</sup> day serum bilirubin (table-2). The test result variable(s): cord blood bilirubin has at least one tie between the positive actual state group and the negative actual state group. Cut off value was 1.95 mg/dL with a sensitivity of 84.8 %, specificity of 82.42%, Positive Predictive Value of 49.12% and Negative Predictive Value of 96.45% (table-3) & (fig -1).

	Mean	S.D.	t	P Value
Cord Bilirubin	1.40	0.72	-45.86	0.001
24 hr Sr. Bilirubin	4.85	1.09		
Cord Bilirubin	1.40	0.72	-75.95	0.001
5th Day Sr. Bilirubin	13.38	2.55		

**Table 2. Comparing the Mean Difference Between the Two Sets of Observations by Paired t Test**

5 <sup>th</sup> Day Jaundice		
Cord Blood	Yes	No
Raised	28	29
Normal	5	136
Total	33	167

**Table 3. Sensitivity, Specificity, PPV, NPV of Cord Blood Bilirubin**

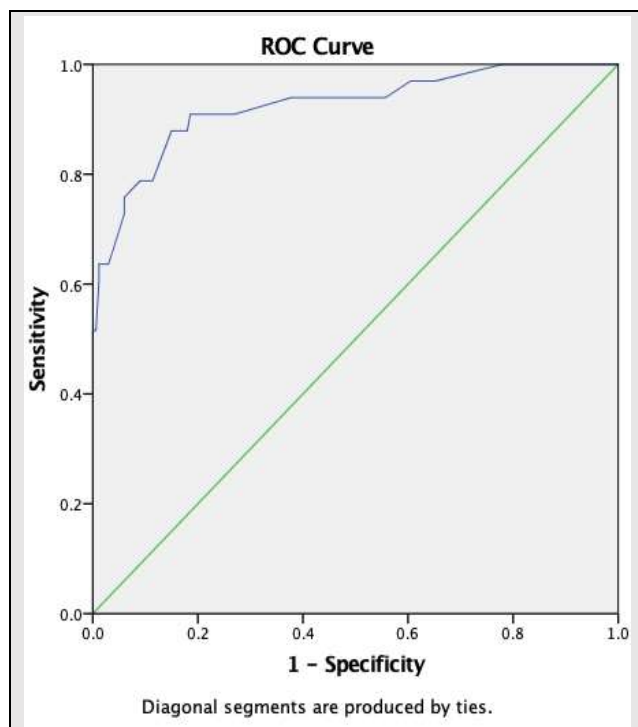


Cut off value for 24 hr serum bilirubin was observed as 5.45 mg/dL by ROC curve with a sensitivity of 87.9%,

specificity of 82%, Positive Predictive Value of 49.15% and Negative Predictive Value: 97.16% (table 4) & (figure 2).

5 <sup>th</sup> Day Jaundice Babies		
24hr Serum bilirubin	Yes	No
Raised	29	30
Normal	4	137
Total	33	167

**Table 4. Sensitivity, Specificity, PPV, NPV of 24 hr Serum Bilirubin**



**Figure 2. ROC Curve Depicting Cut Off Value for 24 hr Serum Bilirubin**

## DISCUSSION

Hyperbilirubinaemia is the most commonly reported cause for readmission during the early neonatal period, and 0.36% of healthy term newborns discharged after 72 hours of life with mild hyperbilirubinemia may even develop subsequent moderate to severe hyperbilirubinaemia.<sup>1</sup> In the united states there were 22 reported cases of kernicterus developing in babies discharged within 48 hours after birth (from 1991 – 1995).<sup>2</sup> Furthermore the safety of relying on follow up visits after early discharge is questionable as 10% of the population fails to return for a follow-up visit.<sup>3,4</sup> In this study it was found that the incidence of significant hyperbilirubinemia to be around 16.5%, which was comparable with the other studies from India, Singhal et al - 5.9%, Narang et al - 6.56% and Maiselsetal USA<sup>1</sup> - 14%.<sup>5,6,7</sup>

In this study the impact of various epidemiological factors over the incidence of jaundice were found. Males were 1:35 times more jaundiced than females in our study. Similar association was demonstrated by Friedman et al, Maisels et al and Anand et al.<sup>8,6</sup> In a study conducted by Alalfy M et al 41 males (54.7%) & 34 females (45.3%) were seen.<sup>9</sup> In a study conducted by Rajpurohit N et al 55% were

males and 45% were females. The male to female ratio was 1.22:1. There was no significant difference in the number of male and female babies.<sup>10</sup> Mode of delivery was vaginal delivery in 62% women, caesarean section in 35% women and forceps delivery in 3% women with the present study. Among jaundiced patients- mode of delivery was vaginal delivery in 54.5% women, caesarean section in 42.4% women and forceps delivery in 3% women. This was supported by a study conducted by Patra LB et al in which Vaginally delivered babies were more prone to develop jaundice later compared to babies born by LSCS.<sup>11</sup> In a study conducted by AlalfyM et al, Vaginally delivered babies were 37.3% and LSCS (62.7%).<sup>9</sup> In a study conducted by Sehgal Pet al Vaginally delivered babies were 38.6% and LSCS (61.4%).<sup>12</sup> Sibling history is present in 11.5% patients. Among jaundiced patients sibling history is present in 15.2% patients. In a study conducted by Patra LB et al exclusive breast feeding and previous sibling with jaundice were not good predictors.<sup>11</sup> Khoury et al demonstrated the impact of jaundice in the sibling over the present child. He has shown that present child has 3 times more risk of jaundice if the previous child has TSB> 12 mg/dL and the risk is 12.5 times if the sibling had TSB> 15 mg/dL.<sup>13</sup>

Intra partum drug oxytocin was administered in 33.5% of the mothers. Among jaundiced patients intra partum drug was administered in 39.4% of the mothers within the present study. Oxytocin has anti-diuretic and natriuretic effects and thus causes hypo-osmolality and hyponatremia in the mother. These biochemical changes are aggravated when oxytocin is given with dextrose solution which was electrolyte free. This hypo-osmolality gets transferred transplacentally to foetus and causes increased red blood cells osmotic fragility. These swollen red blood cells gets easily destroyed in spleen and results in increased bilirubin production.<sup>14</sup> In a study conducted by Rajpurohit N et al, out of total 200 babies, mothers of 30 (15%) babies received oxytocin for the induction of labour and mothers of remaining 170 (85%) babies did not receive oxytocin. Among 30 babies whose mothers received oxytocin 6 (20%) babies developed significant neonatal hyperbilirubinemia while out of 170 babies whose mothers did not receive oxytocin 14 (8.24%) babies developed significant neonatal hyperbilirubinemia. In this study there was significant association between the babies whose mother was given oxytocin for induction of labour and significant neonatal hyperbilirubinemia. Sex, religion, geographical area, birth weight, parity of mother, maternal gestational hypertension, mode of delivery, time of initiation of breast feeding is not associated with significant neonatal hyperbilirubinaemia.<sup>10</sup> Singhi SC, Chookang have found the association between intrapartum oxytocin infusion and increased risk of jaundice as oxytocin can cause haemolysis.<sup>8</sup>

With the present study it was observed that in mothers who were administered oxytocin, high levels of serum bilirubin were found in their babies, but it was not statistically significant, and this was also supported by other studies. In a study conducted by Sneha Taneja, Vineeta Pande et al, no correlation was observed between oxytocin

infusion and neonatal hyperbilirubinemia.<sup>15</sup> In a study conducted by Shai Linn, Stephen C et al, use of oxytocin and neonatal hyperbilirubinemia were not statistically significant.<sup>16</sup> In a study conducted by Engin Oral, Altay Gezer et al, no significant effect of oxytocin infusion was revealed on neonatal hyperbilirubinemia unless oxytocin was for the augmentation of labour.<sup>17</sup> In a study conducted by Ben Wood, Phyllis Culley et al, their results showed no increase in either mean plasma bilirubin levels or of hyperbilirubinaemia whether the oxytocin was given to induce or augment labour.<sup>18</sup>

In the present study, cut off value for cord blood bilirubin was 1.95 mg/dL with sensitivity as 84.8%, specificity as 82.42%, PPV as 49.12%, NPV as 96.45%. Whereas in a study done by Zakianahar et al, in Bangladesh Umbilical cord bilirubin of 2.5 mg/dL had negative predictive value of 96%, positive predictive value of 91%, sensitivity of 77% specificity of 98.59%.<sup>19</sup> Rosenfeld et al, used cord bilirubin >2 mg/dL for predicting significant hyperbilirubinaemia.<sup>20</sup> Knudsen et al found that cord bilirubin level >2.3 mg/dL was associated with increased risk of hyperbilirubinemia.<sup>21</sup> In a study conducted by Rajpurohit N et al, Cord blood bilirubin level of >2 mg/dL had a sensitivity of 90% and specificity of 53.89%, positive predictive value 17.8% and negative predictive value of 98% in predicting the risk of neonatal hyperbilirubinaemia.<sup>10</sup>

In the present study, cut off value for 24 hr serum bilirubin was 5.45 mg/dL with sensitivity of 87.9%, specificity of 82%, PPV was 49.15%, NPV was 97.16%. It was comparable to other studies by Alpay et al and Awasthi et al.<sup>22,23</sup> A Study done by S Randev et al using 1<sup>st</sup> day serum bilirubin for prediction of neonatal jaundice done on 200 neonates, out of which 12% developed hyperbilirubinemia, a value of 6.4 mg/dL (1<sup>st</sup> day TSB) was determined to have best predictive ability for subsequent hyperbilirubinemia with sensitivity 87.5% specificity of 80.11%, positive predictive value of 37.5% negative predictive value of 97.92%.<sup>24</sup>

In the present study, incidence of significant hyperbilirubinemia was 16.5%. In a study conducted by Sourika P et al, the incidence of significant hyperbilirubinemia was 23.7%. Cord serum unconjugated bilirubin level  $\geq$ 2.0 mg/dL and total cord serum bilirubin level  $\geq$  2.5 mg/dL as high risk indicator towards predicting neonatal hyperbilirubinemia in first week of life.<sup>25</sup>

## CONCLUSIONS

There was a statistically significant correlation between 24-hr. serum bilirubin and jaundiced patients and also between 5<sup>th</sup> day serum bilirubin and jaundiced patients. There was a statistically significant correlation between cord bilirubin and 24 hr. serum bilirubin. Thus, cord blood bilirubin and 24 hr. serum bilirubin have role in early prediction of developing significant jaundice in first week of life.

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