



EARLY PREDICTION OF SIGNIFICANT HYPERBILIRUBINEMIA IN PRETERM NEONATES USING 24 HOURS SERUM BILIRUBIN- A PROSPECTIVE STUDY.

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ABSTRACT

Background: Bilirubin induced neurological dysfunction is one of the major causes of morbidity in preterm neonates secondary to uncontrolled hyperbilirubinemia.

While jaundice per se is not preventable none the less early detection of threatening bilirubin levels permit initiation of phototherapy and prevents kernicterus.

Objectives: To determine the first day total bilirubin value, at 24 hours of life; which will predict with reasonable accuracy, preterm neonates likely to develop subsequent significant hyperbilirubinemia requiring treatment.

To establish the cut-off values and comparison of the obtained value for prediction of significant neonatal hyperbilirubinemia in preterm neonates.

Material and methods: The study was conducted on a group of 90 preterm neonates, with no comorbidities, over a period of one year. The main outcome measured was hyperbilirubinemia requiring intervention.

Serum bilirubin level was sent at 24 hours of age. These babies were followed up clinically for the development of jaundice, and subsequent TSB values were obtained and assessed the need of phototherapy.

Results: Mean age at bilirubin estimation was 24 ± 2 hours with mean TSB of 6.03 ± 1.48 mg/dl. Significant hyperbilirubinemia was present in 60/90 babies (66.6%). Neonates belonging to < 32 weeks of gestation age irrespective of their initial TSB values, nearly 100% ended up developing significant hyperbilirubinemia. Whereas in infants belonging to > 32 weeks of gestation age, a TSB level at 24 hours of life, was < 4.0 mg/dl in 4 newborns and none of them developed hyperbilirubinemia subsequently. In the remaining 86 newborns with TSB ≥ 4.0 mg/dl, subsequent hyperbilirubinemia, requiring phototherapy developed in 60 babies (69.8 %) {Sensitivity: 100 %, specificity: 13.3 %, positive predictive value 69.8%, negative predictive value 100% }

Conclusion: Late Preterm babies with TSB levels higher than 4mg/dl at 24 hours of life have a significant risk of developing hyperbilirubinemia.

KEYWORDS : Preterm; Prediction; Hyperbilirubinemia; Kernicterus.

INTRODUCTION

Hyperbilirubinemia also known as “jaundice” is yellowish green pigmentation of the sclera and skin caused by an increase in bilirubin production or a defect in bilirubin elimination. It is one of the most common problems in neonatal period estimated to occur in nearly 60 % of the term infants and 80 % of the preterm infants in the first week of life.¹

Under normal circumstances, the level of indirect reacting bilirubin in umbilical cord serum is 1-3mg/dl and rises at a rate of less than 5mg/dl/24hrs. Thus jaundice becomes visible on the 2nd - 3rd day (36-72hrs) usually peaking by the 3rd day of life. Among preterm babies, age of onset of physiological jaundice is similar to term babies, it may manifest earlier but never before 24 hours of age. The maximum intensity of jaundice is reached on the 5th or 6th day and may persist into 14 days.²

Kernicterus is a rare but devastating condition that is not extinct. It is usually associated with complicating conditions such as iso immunisation or other causes of hemolysis, prematurity, sepsis, other illness or constitutional defects in hepatic bilirubin clearance.³

Quantifying the level of jaundice has been the foundation for satisfactory management of hyperbilirubinemia.

The AAP (American Academy of Paediatrics) recommends⁴ that newborns discharged before or within 48 hours should have a follow-up visit after 2-3 days to detect significant jaundice and other problems.

The American Academy of Pediatrics (AAP) has published a guideline for the management of hyperbilirubinemia in the newborn infant >35 weeks gestation.⁴ But there is no similar universal guidelines published by AAP for preterm infants.

It is generally believed that infants <35 weeks gestation are at greater risk for the development of bilirubin-associated brain damage than term infants, although a paucity of data has made quantification of the magnitude of this risk difficult, and the reported range of bilirubin thresholds used to initiate treatment at different birth weights and gestations is remarkably wide.

The present study was carried out to evaluate the predictive value of specific bilirubin level at 24 hours of postnatal age for identifying preterm neonates at risk for subsequent hyperbilirubinemia.

MATERIAL & METHODS

The present study is a hospital based prospective observational study undertaken to determine the predictive ability of TSB at 24 hours of life for development of significant hyperbilirubinemia, in early post natal days in preterm neonates.

Total of 90 preterm newborns delivered at SDM college of medical sciences and hospital, Dharwad were studied over a period of one year from November 2014 to November 2015.

There was a total of 257 preterm deliveries during the study period. Out of which only 90 preterm newborns were satisfying the inclusion criteria and consented for the study.

Inclusion Criteria: Preterm neonates born at gestation age 28 to 36.6 weeks, who have cried immediately at birth, haemodynamically stable with no significant co morbidities like respiratory distress, metabolic abnormalities, sepsis, apnoea etc.

Exclusion criteria (make a paragraph all the criteria)

Gestational age > 37 weeks

Rh/ & ABO incompatibility

Babies with birth asphyxia

Babies with sepsis screening positive

Babies with congenital malformations (tracheoesophageal fistula, anorectal malformations, etc)

Babies with cephalhematoma and birth injury.

Babies with maternal intake of drugs affecting maternal liver-sulphonamides, nitrofurantoin, anticonvulsants etc.

Babies with PPROM > 18 hours.

Methodology. Preterm babies satisfying the inclusion criteria with detailed maternal history, gestational age assessed by New Ballard Scoring system was investigated for serum bilirubin levels at 24 hours of life. These babies were clinically followed up for the appearance of

jaundice and thus subsequent serum bilirubin levels were obtained in those babies who developed jaundice. Babies with significant hyperbilirubinemia were treated with phototherapy according to their gestational age. The obtained values and observations were statistically analysed and a cut off value at 24 hours of life was obtained, which could predict the occurrence of significant hyperbilirubinemia.

Laboratory evaluation (Bilirubin estimation).

Sample Required : 2 ml venous blood sample. Serum bilirubin estimation was done using the modified "Jendrassik and Grof" method. Whole blood was taken in micro-capillary and centrifuged at the rate of 3000 rpm for 5 minutes. Serum bilirubin estimation was done using the Bichromatic wavelength 540nm designed for the automated device Siemens- Dimensions RL Max. TSB was estimated by above method at 24 hours of life. The neonates were followed up clinically every 12 hrs for the development of jaundice. Subsequent Bilirubin estimation was done whenever clinical appearance of jaundice was present.

TABLE 1 : Use of phototherapy and exchange transfusion in preterm infants < 35 weeks of gestational age. (this table is not required but you can put a reference number quoting we used this table for treatment in preterm infants)

Use of phototherapy and exchange transfusion in preterm infants < 35 weeks yes		
gestational age (weeks)	initiate phototherapy TSB (mg/dl)	exchange transfusion TSB (mg/dl)
< 28 0/7	5-6	11-14
28 0/7 - 29 6/7	6-8	12-14
30 0/7 - 31 6/7	8-10	13-16
32 0/7 - 33 6/7	10-12	15-18
34 0/7 - 34 6/7	12-14	17-19

management protocol as per gestational age published by Maisels et al as given in table 1.⁴ For babies more than 35 weeks of gestation, the hour specific bilirubin nomogram published by the AAP was used to assess the significant hyperbilirubinemia and need of phototherapy.⁴

Statistical Analysis

Sensitivity, specificity, negative and positive predictive values of the test were calculated using the SSPE software. For determining significance of each test p value was used. Significance was also established by the ROC curve.

RESULTS & OBSERVATIONS

Out of the total 90 babies, 60 babies developed significant hyperbilirubinemia requiring phototherapy, 30 babies did not.

With the increase in level TSB at 24 hours has a definite association with the risk of developing significant neonatal jaundice in the subsequent post natal days, with p value being highly significant.

In our study there is no significance between initial value of TSB and subsequent hyperbilirubinemia in the babies < 32 weeks of gestation.

Babies belonging to gestation age 32.1 to 34 weeks, and 34.1 to 36.6 weeks, 58.8 % and 33.3% developed significant hyperbilirubinemia respectively, corresponding with increasing trends of initial first day value, thus p value being < 0.05 and 0.009 respectively.

Based on our frequency table and trend of occurrence of jaundice in babies >32 weeks of gestation age, an arbitrary value of 4 mg/dl was considered as a cut off value at 24 hours of life to predict the development of significant jaundice in the subsequent post natal days. Out of the 90 babies, 60 babies who developed jaundice and 26 babies who did not require phototherapy had values > 4mg/dl. This test of evaluating a TSB level at 24 hours of life, and predicting if these babies will develop significant jaundice with a cut off value of 4 mg/dl had a PPV of 69.8 % and NPV of 100 %.

The hypothesis that TSB at 24 hours of age, can predict the occurrence of significant hyperbilirubinemia, was proved to be significant for the babies >32 weeks of gestation age, by using ROC curve, where in the area under the curve being 0.541 and is statistically significant.

We could observe from our frequency tables, that a TSB in the range of

4-5 mg/dl and greater have found to show the development of significant hyperbilirubinemia. Thus based on our frequency table, considering 4 mg/dl at 24 hours of life as an arbitrary cut off value the sensitivity specificity PPV and NPV were calculated as follows. The results shows that the test is 100 %, specificity 13.3 % and PPV of 69.8%, NPV of 100 %, indicating that there is high chance of developing subsequent hyperbilirubinemia requiring phototherapy if these babies after 32 weeks of gestation age have an initial TSB level of >4.01 mg/dl at 24 hours of life.

TABLE NO 2: ASSOCIATION OF MATERNAL RISK FACTORS WITH THE DEVELOPMENT OF SIGNIFICANT HYPERBILIRUBINEMIA.

Maternal Complications	Jaundice requiring phototherapy		Total
	Present	Absent	
Absent	4(36.4)	7(63.6)	11
Present	56(70.9)	23(29.1)	79
Total	60(66.7)	30(33.3)	90

PVALUE = 0.023 (significant)

FIGURE 1 : JAUNDICE AT 24 HOURS OF LIFE, FOR ALL THE TOTAL NEONATES ENROLLED IN THE STUDY (This figure is not required in publication)

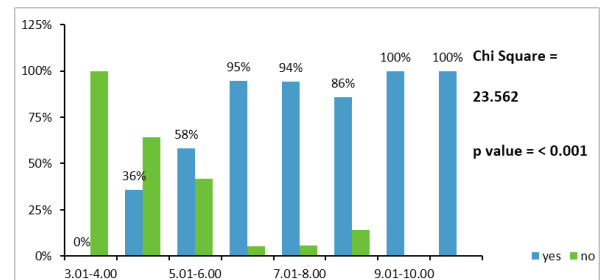


TABLE NO 3 : JAUNDICE REQUIRING PHOTOTHERAPY BASED ON GESTATIONAL AGE

Gestation age	Jaundice requiring phototherapy		Total
	present(%)	absent(%)	
28.1-30 weeks	6(100)	0	6
30.1- 32 weeks	27(93.1)	2(6.9)	29
32.1- 34 weeks	20(58.8)	14(41.2)	34
34.1- 36.6 weeks	7(33.3)	14(66.7)	21
Total	60(66.7)	30(33.3)	90

TABLE NO 4 : TSB AT 24 HOURS OF LIFE IN GESTATION AGE GROUP 28 TO 30.6

TSB 24hrs(mg/dl)	Jaundice requiring phototherapy present(%)
2.01-3.00	2(100)
4.01-5.00	2(100)
>5.01	2(100)
Total	6 (100)

We notice that there were only 6 babies included in this category, and all of them have developed jaundice requiring phototherapy. Thus no p value could be obtained.

TABLE NO 5 : DEVELOPMENT OF SIGNIFICANT HYPERBILIRUBINEMIA IN GESTATION AGE 30.1 TO 32 WEEKS

TB at 24 hrs(mg/dl)	Jaundice requiring phototherapy		Total
	present(%)	absent(%)	
4.01-5.00	4(80)	1(20)	5
5.01-6.00	4(80)	1(20)	5
6.01-7.00	8(100)	0(0)	8
7.01-8.00	6(100)	0(0)	6
8.01-9.00	4(100)	0(0)	4
9.01-10.00	1(100)	0(0)	1
Total	27	2	29

Chi square = 4.081 p value = 0.538

TABLE NO 6 : DEVELOPMENT OF SIGNIFICANT HYPERBILIRUBINEMIA IN GESTATION AGE 32.1 TO 34 WEEKS

TB at 24 hrs(mg/dl)	Jaundice requiring phototherapy		Total
	present(%)	absent(%)	
3.00-4.00	0(0)	2(100)	2
4.01-5.00	2(20)	8(80)	10
5.01-6.00	3(60)	2(40)	5
6.01-7.00	8(100)	0(0)	8
7.01-8.00	5(83.3)	1(16.7)	6
8.01-9.00	1(50)	1(50)	2
>10.01	1(100)	0(0)	1
Total	20(58.8)	14(41.2)	34

Chisquare=16.935 p value=0.01 (significant)

TABLE NO 7 : DEVELOPMENT OF SIGNIFICANT HYPERBILIRUBINEMIA IN GESTATION AGE 34.1 TO 36.6 WEEKS

TB at 24 hrs (mg/dl)	Jaundice requiring phototherapy		Total
	present (%)	absent (%)	
3.01-4.00	0(0)	2(100)	2
4.01-5.00	2(18.2)	9(81.8)	11
5.01-6.00	0	2(100)	2
6.01-7.00	0	1(100)	1
7.01-8.00	4(100)	0	4
8.01-9.00	1(100)	0	1
Total	7(33.3)	14(66.7)	21

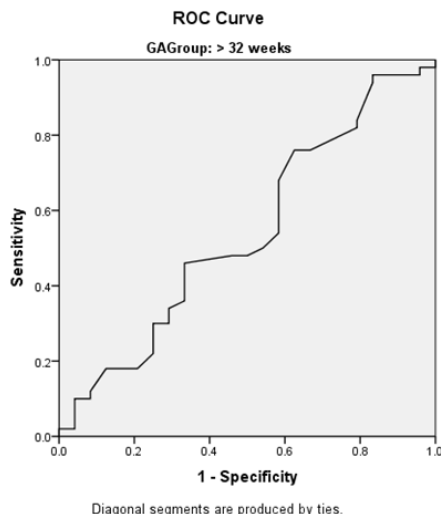
Chisquare=11.66 p value=0.009 (significant)

TABLE NO 8 : RESULTS FOR PREDICTIVE VALUE OF 4 mg/dl TSB AT 24 HOURS OF LIFE

TSB at 24hrs	Developed jaundice	Did not develop jaundice
< 4.00mg/dl	0	4
>4.01mg/dl	60	26

Sensitivity = 100% Specificity = 13.3%

Positive Predictive value = 69.8% Negative Predictive value = 100%

Figure 1 : ROC curve for GA>32 weeks, TB at 24 hours may be used as a predictor of significant jaundice. AUC=0.541

DISCUSSION

In the last 10 to 20 years we have witnessed autopsy proven kernicterus. This has been reported in premature infants, belonging to very low birth weight and sick babies; and also a few cases with low TSB levels [low bilirubin kernicterus].^{6,7,8} Current studies done by Govaret P et al ,Moll M et al and Sugama et al have documented that this still remains a clinical threat.⁹⁻¹¹

Unlike the above mentioned studies, where in kernicterus was found in sick neonates ; in another study, two healthy preterm neonates with uneventful NICU stay of 31 and 34 weeks gestation age, developed

choreoathetosis and at follow up had clinical features of kernicterus and both the babies peak TSB levels were 13.1 mg/dl and 14.7 mg/dl respectively.¹¹

Recent studies of large populations of ELBW infants suggest an association between neurodevelopment impairment and modest elevations in TSB.^{12,13}

So therefore it is of utmost importance to derive a predicting value to prevent the untoward consequences. From various studies conducted on term, healthy babies we know the fact that neonates who are clinically jaundiced in the initial few days are at risk of developing significant hyperbilirubinemia in subsequent post natal days.

In our study babies developing significant hyperbilirubinemia is much higher as compared to studies done on term neonates, like Aplay et al¹⁴ ; Knuffer et al;¹⁵ Awasthi et al.¹⁶ The higher percentage found in our study on these preterm babies may be attributed to their inability to handle bilirubin load, decreased hepatic UDP glucuronyl transferase enzyme activity, and a slower post natal maturity of hepatic bilirubin uptake, leading to early hyperbilirubinemia and also a lesser threshold of requirement of phototherapy as per standard guidelines to prevent bilirubin related neurological complications in these vulnerable neonates. And several previous by Bhutani et al¹⁷ and Seidman et al,¹⁸ have applied the same methodology and found the association between bilirubin levels in cord blood and subsequent risk of hyperbilirubinemia.

However most of these studies have been done on term babies and thus poor data is available on preterm neonates to support the above mentioned association.

We observed that with the increase in level TSB at 24 hours has a definite association with the risk of developing significant neonatal jaundice in the subsequent post natal days. The neonates who did not develop significant jaundice has gradually decreased with the increase in initial TSB levels. And the results are statistically highly significant with p value being <0.001.

And all these babies with significant hyperbilirubinemia required only phototherapy and none of them were in the exchange transfusion range.

Effective phototherapy dramatically decreases the need for exchange transfusion in preterm infants, so that the procedure has become increasingly rare in the NICU.

The significant findings in our study for neonates belonging to 32 weeks and more of gestational age are in agreement with Indian study done by Lavanya et al¹⁹ on 216 late preterm infants, which has concluded that bilirubin levels measured at 24-48 hours of life better predicts 'significant jaundice after 48 hours of life, in comparison with clinical risk factors.

A study done by Izi Mayer et al.²⁰ at an NICU in Istanbul , on total of 150 preterm neonates, born ≤ 35 weeks, were assessed for TSB on first day at 12 th hours of life and subsequently followed up . However unlike our study the babies were categorised based on their birth weight into two categories and was found that capillary billrubin levels of 3.55 mg/dl and 4.55 mg/dl for birth weight 1 to 1.5 kg and 1.5 to 2 kg group respectively at 12 th hour of life had the highest sensitivity , NPV and PPV for the prediction of subsequent significant hyperbilirubinemia.

LIMITATIONS

1. In this study we were unable to derive a predictive value and significance of initial TSB levels in determining the occurrence of subsequent significant hyperbilirubinemia in < 32 weeks of gestation age, this requires a large group of study population to derive at a definite conclusion.(this limitation can be deleted)
2. We have not screened for G6PD deficiency, as the incidence and prevalence of G6PD In Indian population is rare.
3. We have not ruled out minor blood group abnormalities as laboratory facilities for the same was not available in our institution.
4. Among the study population, few babies were on IV fluids, TPN and few babies on feeds, due to their requirements based on gestational age. Therefore uniformity could not be maintained in

this aspect.

5. Clinical detection of jaundice has observer variation and is subjective. (this limitation can be deleted)

CONCLUSION

Preterm babies are a group of high risk and vulnerable individuals for developing bilirubin induced neuronal dysfunction, requiring early intervention. Therefore our study has helped us to understand the use of routine screening for TSB on the first day of life. Late Preterm babies with TSB levels higher than 4mg/dl at 24 hours of life have a significant risk of developing hyperbilirubinemia. This will definitely help in predicting the babies at risk of developing hyperbilirubinemia and preventing bilirubin related complications, if intervened at the appropriate time.

However further more exploration and research is needed on a larger population to form an uniform consensus guidelines for this topic.

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