

CORD BILIRUBIN VALUE AS A PREDICTOR OF SIGNIFICANT HYPERBILIRUBINEMIA IN ABO INCOMPATIBILITY

ABSTRACT

Background: Hyperbilirubinemia is a condition in which the blood contains too much bilirubin and producing jaundice (yellow coloring of the eyes and skin). Low bilirubin levels in newborns are common and do not pose any problems; they will resolve on their own within the first week of life. Studying the cord bilirubin levels in new born babies is significant to predict the risk of abo incompatibility

Methods: A total of 129 babies born to O blood group mother were included in the study. Out of which 111 babies were with risk of ABO incompatibility. Among them 17 babies developed pathological hyperbilirubinemia. None of the O positive babies developed pathological hyperbilirubinemia.

Results: The peak bilirubin level was attained on 3rd and 4th day for all the babies and was taken as the outcome measure and cord serum bilirubin was taken as the predictive factor. The incidence of pathological hyperbilirubinemia is 13.2%. The mode of delivery had no positive association with the development of pathological hyperbilirubinemia. Male babies had positive association for pathological hyperbilirubinemia without any statistical significance. Incidence of pathological hyperbilirubinemia is higher in babies with a birth weight of <3 kg.

Conclusion: A cord bilirubin value of 2.65mg/dL can be used as a cut off for predicting pathological hyperbilirubinemia. Infants with bilirubin level more than the cutoff values were subjected to early intervention with complete recovery. None of the babies had developed encephalopathy and its sequelae.

Keywords: Newborn, O Blood group mother, Jaundice, hyperbilirubinemia.

1. INTRODUCTION

Jaundice is the visible manifestation in skin and sclera of elevated serum concentration of bilirubin. Jaundice is a common finding in first week of newborn period. The clinical jaundice will manifest in neonates at a serum bilirubin level above 5.0 to 7.0 mg/dl (86-119 micromoles/L). Traditionally, there has been a difference drawn between benign physiological hyperbilirubinemia and hyperbilirubinemia that is either pathogenic in origin or severe enough to need further evaluation and management. This latter condition is referred to as "pathological jaundice." If the concentration of serum total bilirubin surpasses 10mg/dl on the first day of life in a term newborn, 10mg/dl on the second day, or 12 to 13 mg/dl thereafter, it is considered non-physiological [1,2]. Hemolytic disease of newborn is one among the causes of hyperbilirubinemia. Since the introduction of Rh immunoglobulin as a treatment for Rh isoimmune hemolytic disease of newborn, The common blood group incompatible hemolytic process of the newborn period is ABO hemolytic illness. Although fetal-maternal ABO incompatibility occurs in around a quarter of all pregnancies, hemolytic illness affects just one out of every ten children [3].

ABO hemolytic illness can strike at any time during pregnancy, including the first, but it is only passed down to group A and group B babies delivered to group O women. The impact of maternal anti A or anti B antibodies on foetal erythrocytes of the same blood group causes ABO hemolytic disorder. In the same way that maternal anti A or anti B antibodies enter foetal circulation and react with A or B antigen on erythrocyte surface, hemolysis associated with ABO incompatibility is analogous to Rh hemolytic disorder. Anti-B and anti-C antibodies are normally present in type A and B people. Anti B and anti A isoantibodies are mostly Ig M

molecules that do not pass the placenta. The alloantibodies seen in type O people, on the other hand, are mostly Ig G molecules. As a result, ABO incompatibility is mostly limited to type O women with type A or B fetuses [3,4]. Discharge of newborn from hospital, newborn with ABO incompatibility are at especially greater risk for developing a subsequent significant hyperbilirubinemia and subjected to re-admission for phototherapy or exchange transfusion treatment. Such re-admissions, besides involving extra expenses for both the family and the institution and also exposing a probably healthy newborn to the hospital environment, risks to breast-feeding and is one of the causes of early weaning [5-8]. Already many studies are in pipeline for risk factors like Rh incompatibility, G6PD deficiency, but less number in ABO incompatibility. So studies regarding estimation and correlation of cord bilirubin with significant hyperbilirubinemia in ABO incompatibility will be useful [9].

MATERIALS AND METHODS

STUDY DESIGN Prospective observational study

STUDY PERIOD & PLACE: August 2010 - August 2011. Sree Balaji Medical College and Hospital, Chromepet, Chennai

STUDY POPULATION: Babies born of 'O' positive mothers were randomly selected for the study. Ethical approval for this study was obtained from Institutional review board (IEC/C-P/126/2018).

OUTCOME MEASURES: Pathological hyperbilirubinemia was defined in our study as a total blood bilirubin level > 15 mg/dL on the fourth day of life or any total serum bilirubin level greater than the 95th percentile for the age in hours.

SAMPLE SIZE: The sample size was determined by the frequency of pathological bilirubinemia in ABO incompatibility. The sample size was calculated to be 129 for a correlation of 0.6, alpha error of 0.0, and beta error of 0.01.

INCLUSION CRITERIA:

- 1) Babies born with A or B or AB or O blood group born to O positive mothers
- 2) New born of GA 37 weeks.
- 3) New born of B.wt 2.5 kg and 4 kg.
- 4) Apgar > 7 in new born at birth

EXCLUSION CRITERIA:

- 1) Neonatal problems causing hyperbilirubinemia like prematurity, birth asphyxia, birth Trauma like cephalhaematoma, sepsis, hypothyroidism and congenital malformation.
- 2) Significant disease in mother, which can cause hyperbilirubinemia in newborn, like gestational diabetes mellitus and gonorrhoea.

PROCEDURE:

The research took place at Sree Balaji medical college and hospital in Chromepet, Chennai. Babies that met the following criteria were chosen at random for the study. Following the baby's initial stabilization, 3 milliliters of cord blood was obtained in two separate prepared bottles and promptly sent to the laboratory for blood grouping, Rh typing, and serum bilirubin.

A detailed new born information and medical history was collected, with a focus on the circumstances that lead to pathological hyperbilirubinemia. Neonatal problems included delayed meconium passage, delayed feeding, and inadequate feeding. The weight of the baby at birth was recorded. A thorough general examination was performed to rule out congenital

malformations and hidden haemorrhage such as cephalhematomas. The study eliminated those babies who had any additional risk factors besides ABO incompatibility.

ABO incompatibility was found in 111 of the 129 newborns who were examined (A or B or AB group babies delivered to O group mother). The remaining 18 infants belonged to the O group. All babies with the letters A, B, AB, or O were checked twice a day for clinical jaundice. If the baby developed clinical jaundice, the serum bilirubin level was determined and the proper medication was administered.

ESTIMATION OF SERUM BILIRUBIN

The Diazo technique was used to calculate bilirubin at our biochemistry department. Absolute methanol, hydrochloric acid, diazo reagent, and a standard bilirubin solution were utilised as reagents. The serum was diluted with water, and methanol was added in an amount that was not enough to precipitate proteins but enough to assure that all of the bilirubin was reacting with the diazo reagent. Bilirubin interacts with diazotized sulphanilic acid to form azo bilirubin, which may be measured via spectroscopy.

RESULT

The total number of deliveries in our hospital, during the study period from August 2010 to August 2011 was 432. 129 babies were born to O blood group mother were included in the study. Out of which 111 babies were at risk of ABO incompatibility and remaining 18 of them had O blood group.

The data obtained was **analyzed** as follows:

1. Incidence of physiological and pathological hyperbilirubinemia.
2. Distribution of physiological and pathological hyperbilirubinemia according to mode of delivery, sex, birth weight and blood group.
3. Correlation of cord blood bilirubin value with pathological hyperbilirubinemia.
4. Receiver operated characteristic curve for cord bilirubin and cut off value for predicting pathological hyperbilirubinemia.
5. Treatment and immediate outcome of infants with pathological hyperbilirubinemia.

Table 1. Incidence of physiological and pathological hyperbilirubinemia (n=129).

| No jaundice | Physiological hyperbilirubinemia | Pathological hyperbilirubinemia |
|-------------|----------------------------------|---------------------------------|
| 58 | 54 | 17 |

Out of the 129 babies, 54(42%) of the babies developed physiological hyperbilirubinemia and 17(13%) of cases developed pathological hyperbilirubinemia.

Table 2. Mode of delivery distribution in babies who developed no jaundice, physiological hyperbilirubinemia, pathological hyperbilirubinemia (n=129)

| Delivery | No jaundice | Physiological hyperbilirubinemia | Pathological hyperbilirubinemia |
|----------|-------------|----------------------------------|---------------------------------|
| Normal | 25 | 28 | 9 |

| | | | |
|---------------------|----|----|---|
| Instrumental | 6 | 3 | 0 |
| LSCS | 27 | 23 | 8 |

Out of 129 babies 28 (21.7%) delivered by normal delivery and 23 (17.82%) delivered by LSCS developed physiological hyperbilirubinemia. 9 (6.97%) delivered by normal delivery and 8 (6.20%) delivered by LSCS developed pathological hyperbilirubinemia. Physiological and pathological hyperbilirubinemia did not show any statistical difference among mode of deliveries.

Table 3. Sex distribution in babies who developed No jaundice, physiological hyperbilirubinemia, pathological hyperbilirubinemia (n=129).

| Sex | No jaundice | Physiological hyperbilirubinemia | Pathological hyperbilirubinemia |
|--------|-------------|----------------------------------|---------------------------------|
| Male | 28 | 25 | 11 |
| Female | 30 | 29 | 6 |

The incidence of pathological hyperbilirubinemia was more in male 11 (8.52%) when compared to female 6 (4.65%), but there was no statistically significant difference (p-value 0.182)

WEIGHT DISTRIBUTION

In our study group babies with birth weight of 2.5 to 4 kilograms were only included (Appropriate for Gestational age) (n=129)

Table 4. Weight distribution in babies who developed no jaundice, physiological hyperbilirubinemia, pathological hyperbilirubinemia(n=129).

| Wt (kg) | No Jaundice | Physiological hyperbilirubinemia | Pathological hyperbilirubinemia |
|---------|-------------|----------------------------------|---------------------------------|
| 2.5-3 | 38 | 34 | 12 |
| 3.1-3.5 | 19 | 15 | 4 |
| 3.6-4 | 1 | 5 | 1 |

The incidence of pathological jaundice (n=7) for birth weight of 2.5-3 kg is 12 (70.6%), 3.1-3.5kg is 4 (23.5%) and 3.6-4 kg is 1(5.9 %). If birth weight is < 3kg incidence of pathological jaundice is found to be higher compared to birth weight of > 3kg. In order to confirm this ANOVA test has been done, which provided statistically significant F value of 74.84 with (P-value of 0.000)

BLOOD GROUP AS A RISK FACTOR

Table 5. Blood group distribution in babies who developed no jaundice, physiological hyperbilirubinemia, pathological hyperbilirubinemia .(n=129)

| Blood group | No Jaundice | Physiological hyperbilirubinemia | Pathological hyperbilirubinemia |
|-------------|-------------|----------------------------------|---------------------------------|
| A+ | 13 | 26 | 9 |
| A- | 4 | 0 | 0 |
| B+ | 20 | 21 | 8 |
| B- | 2 | 0 | 0 |
| AB | 3 | 5 | 0 |

| | | | |
|---|----|---|---|
| 0 | 16 | 2 | 0 |
|---|----|---|---|

Out of the 129 babies, 52 were A group (48 had A positives group and 4 had A negatives group), 51 were B group (49 had B positives group and 2 had B negative group), 8 were AB positive group and 18 were O positive group. Incidence of physiological hyperbilirubinemia was higher in A group 26(20.15%) when compared to B group 21(16.27%) (p-value 0.006). No significant difference in incidence of pathological hyperbilirubinemia was noticed between group A and group B (p-value 0.523). None of the baby in O group developed pathological hyperbilirubinemia.

Table 6. Association of cutoff value of cord bilirubin value with pathological hyperbilirubinemia.

| Chord bilirubin(mg/dl) | Pathological jaundice | |
|------------------------|-----------------------|---------------|
| | Present (n=17) | Absent (n=12) |
| >2.65 | 16 | 31 |
| <2.65 | 1 | 81 |

The cutoff value of 2.65 mg/dl was tested for significance with chi square test and found to be 30.39, (p-value 0.000) with odds ratio 45.9 with 95% CI (5.8-360.06) which means that babies with cord bilirubin value >2.65 mg/dl had 45 times higher risk of developing pathological jaundice.

Table 7. Details of phytotherapy treatment to 17 babies with pathological hyperbilirubinemia

| Pathological Jaundice | Phototherapy | Exchange Transfusion |
|-----------------------|--------------|----------------------|
| 17 | 16 | 1 |

All 17 babies with pathological hyperbilirubinemia received phototherapy out of which one baby who had serum bilirubin of 18.2 on day 2 of life required exchange transfusion. All babies recovered without any complications.

Table 8. Short term outcome

| Outcome | Pathological jaundice (n=17) | % |
|-----------|------------------------------|------|
| Discharge | 17 | 100% |
| Expired | 0 | 0 |

DISCUSSION

ABO incompatibility is the most common cause of pathological hyperbilirubinemia.' The goal of this study was to see if bilirubin analysis in cord blood could be beneficial in predicting pathological hyperbilirubinemia in neonates with ABO incompatibility. This study comprised 129 kids born to O positive women with blood groups A, B, AB, or O. ABO incompatibility was a concern for 111 newborns. All of the babies were born at a healthy gestational age (>37 weeks) (birth weight of 2.5 - 4 kg). Babies with various risk factors for jaundice, such as bilih asphyxia, birth injury, sepsis, and mothers with diaper rash [10-12].

Pathological hyperbilirubinemia was defined as a 4th day bilirubin value greater than 15 mg/dL or a serum bilirubin level greater than the 95th centile for the age in hours in our study. Pathological hyperbilirubinemia developed in 17 of 111 newborns who were at risk of ABO incompatibility. Pathological hyperbilirubinemia did not occur in any of the newborns with the O blood group. [13-14].

Incidence of pathological hyperbilirubinemia

Incidence of pathological hyperbilirubinemia in various studies range from 3.7% to 32.95% [15]. In our study incidence of pathological hyperbilirubinemia was 13.1%.

Sex and hyperbilirubinemia

Earlier studies have shown that male gender as a risk factor for hyperbilirubinemia [16]. However our study has showed only a positive association which is not statistically significant.

Birth weight and hyperbilirubinemia

Incidence of pathological jaundice was found to be higher when birth weight was < 3kg when compared to birth weight > 3kg (p-value 0.000)

Peak bilirubin levels

Peak bilirubin is generally attained between 3rd and 4th day in term infants [17]. In our study also, bilirubin peaking was mostly noted on the 3rd and 4th day.

The mean bilirubin level in babies with pathological hyperbilirubinemia was 3.7 mg/dL and in babies with physiological hyperbilirubinemia was 2.4mg/dl, whereas the mean cord bilirubin in babies who had no jaundice was 2.1 mg/dL (P-value <0.001).

Correlation of cord blood bilirubin with hyperbilirubinemia

Previous studies conducted by the authors showed a good correlation between cord blood bilirubin and the development of pathological hyperbilirubinemia [16]. In our study cord bilirubin has excellent correlation with the 4th day bilirubin levels. Pearson's correlation, $r=0.71$ (p -value <0.000). So, cord bili' in can effectively predict the risk of pathological hyperbilirubinemia.

Cord bilirubin value as predictor for pathological hyperbilirubinemia

According to our study cord bilirubin value of 2.65mg/dL can be used as a cut-off for predicting pathological hyperbilirubinemia with a specificity of 73%, sensitivity of 94.4%, chi-square value 30.39(p -value 0.000)

Phototherapy:

All 17 babies who had pathological hyperbilirubinemia received phototherapy and 1 of them required exchange transfusion. The decision to start and continue phototherapy was based on normogram for serum bilirubin. All the 17 babies who required phototherapy recovered completely and were discharged.

Studies regarding early identification of newborns at risk of hyperbilirubinemia at birth are inadequate and more studies are needed in forth coming years. There are many ongoing studies of identifying newborns at risk like cord bilirubin, cord blood hemoglobin concentration, cord blood reticulocyte count without any statistically proven guidelines.

CONCLUSION

The value of cord bilirubin can be used to predict pathological hyperbilirubinemia in babies who are at risk of ABO incompatibility. Higher cord bilirubin levels are associated with higher blood bilirubin levels. Male babies are more likely to have pathological hyperbilirubinemia. Babies with a birth weight of < 3 kg are at a higher risk of developing pathological hyperbilirubinemia. Neonates with cord bilirubin values 2.65mg/dL are at higher risk for developing pathological hyperbilirubinemia. Neonates who received appropriate interventions recovered without any morbidity or mortality. Neonates with cord bilirubin value >2.65 mg/dl should be closely monitored for hyperbilirubinemia. This will help in early identification and treatment of at risk newborns and hence prevent bilirubin encephalopathy.

REFERENCE:

1. Pradhan A, Lamichaney R, Sharma V. Cord blood bilirubin level as a predictor of development of pathological hyper bilirubinemia in new-borns. *Int J Contemp Pediatric* 2017; 4: 1519-1524.
2. Bhutani VK, Johnson-Hamerman L. The clinical syndrome of bilirubin-induced neurologic dysfunction. *Semin Fetal Neonatal Med.* 2015; 20(1): 6-13.
3. American Academy of Pediatrics Subcommittee hyper bilirubinaemia. Management of hyper bilirubinaemia in the newborn infant 35 or more week of gestation. *Pediatrics* 2004; 114(1): 297-316.
4. Le LT, Partridge JC, Tran BH, Le VT, Duong TK, Nguyen HT, Newman TB. Care practices and traditional beliefs related to neonatal jaundice in northern Vietnam: a population-based, cross-sectional descriptive study. *BMC Pediatr.* 2014; 14: 264.
5. Habib HS. Impact of discharge timings of healthy newborns on the rates and etiology of neonatal hospital readmissions. *J Coll Physicians Surg Pak.* 2013; 23(10): 715-719.

6. Vasudevan J, Reddy GMM, Thayumanavan S. Usefulness of cord bilirubin as a screening test to predict newborns at risk of hyperbilirubinemia. *Pediatric Oncall*. 2013; 10: 9.
7. Taksande Amar, Vihekar Krishna, Manish, et al. Prediction of the development of neonatal hyperbilirubinemia by increased umbilical cord blood bilirubin. *Indmedica Curr Pediatric Res* 2005; 9(1): 1–9.
8. Nahar Z, Shahidullah MD, Mannan A, Dey SK, Mitra U, Selimuzzaman 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(2): 50-54.
9. Bernaldo AJ, Segre CA. Bilirubin dosage in cord blood: could it predict neonatal hyperbilirubinemia? *Sao Paulo Med J*. 2004; 122(3): 99-103.
10. BhutaniVK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial pre-discharge newborn population to assess the risk of severe hyperbilirubinemia. *Pediatrics*. 2000; 106(2): E17.
11. Lock M, Ray JG. Higher neonatal morbidity after routine early hospital discharge: Are we sending newborns home too early? *Can Med Assoc J* 1999;161(3): 249.
12. Chary E, Bharadwaj N, Kumar P, Vivekanand N, Sailaja V, Harika B. Umbilical Cord Blood Bilirubin Level Measurement in Predicting the Development of Significant Hyperbilirubinemia. *Indian Journal of Mednodent and Allied Sciences*. 2014; 2(2) :144.
13. Ahire N, Sonawane R, Gaikwad R, Patil S. Study of Correlation of Cord Blood Bilirubin with Neonatal Hyperbilirubinemia. *MVPJOM* 2016; 3: 1.
14. Rajpurohit N, Kumar S, Sharma D, Choudhary M, Purohit S. To Assess Predictive Value of Cord Blood Bilirubin and Albumin for Significant Neonatal Hyperbilirubinemia: A Prospective Study from India. *J Pediatric Neonatal Care* 2015; 2(1): 00060.
15. Han P, Kiruba R, Ong R, Joseph R, Tan KL, Wong HB. Haemolytic disease due to ABO incompatibility: incidence and value of screening in an Asian population. *Aust Paediatr J*. 1988; 24(1): 35-38.
16. Procianny RS, Giacomini CB, Farina DM, Mollin GA, Winckler MI, Silveira MB, Campos L, Marques-Pereira JP. Early diagnosis of ABO haemolytic disease of the newborn. *Eur J Pediatr* 1987; 146(4): 390-393.
17. Madan A, James R, Stevenson DK. Neonatal hyperbilirubinemia. *Avery's Disease of the Newborn*. 2016; 5(1): 1226-1256.