INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Correlation Of Cord Blood Bilirubin And Neonatal Hyperbilirubinemia In Newborns With A Setting Of Abo Incompatibility

Pooja Patel[1], Kirankumar Angadi[2], Pratyush Gupta[3], Rahul Jain[3], Priyanshu Saraswat[3], Karan Chauhan[3], Himani Jain[3]

[1]Assistant Professor, Department of Pediatrics, Smt. B.K.Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth (An institution deemed to be university), Piparia, Vadodara, India

[2]Senior Resident Doctor, Department of Pediatrics, Smt. B.K.Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth (An institution deemed to be university), Piparia, Vadodara, India

[3]2nd year Residents Doctor, Department of Pediatrics, Smt. B.K.Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth (An institution deemed to be university), Piparia, Vadodara, India

Abstract

Background: Neonatal Hyperbilirubinemia (NH) is commonest benign physical finding during the first week of life which require evaluation and treatment. In significant number (6.5%) of newborns, Neonatal Hyperbilirubinemia (NH) is also the most common cause for readmission during the early neonatal period. Umbilical cord bilirubin measurement is a cheap, non invasive, readily available investigation that can help in predicting the development of hyperbilirubinemia in neonates. The aim of this study is to study the correlation of Cord blood bilirubin level with subsequent neonatal hyperbilirubinemia with a setting of ABO incompatibility in neonates.

MATERIALS AND METHOD: 324 healthy term inborn neonates with A, B, or AB blood group born to mother s with O blood group were enrolled in the study, during study period of one and half year. Cord blood was collected from the placental side of cord. Cord and blood bilirubin were estimated. Also Serum Bilirubin (total and indirect) was done from peripheral blood at 24 hours, 48 hours and 72 hours of life.

RESULTS: Incidence of significant hyperbilirubinemia in the study at 24 hours of life was 13%. Mean Serum Bilirubin at postnatal 72 hours of life was 11.1 gm/dl. Using cord blood bilirubin level of > 3 mg/dl as cut-off value, neonatal hyperbilirubinemia can be predicted with sensitivity of 85%, specificity of 93.5%, positive predictive value of 69% and negative predictive value of 98%.

CONCLUSIONS: In the present study a Negative Predictive Value of 98% suggests that in Healthy Term babies with ABO incompatibility having a CBB <3 mg/dl, are unlikely to require further evaluation and intervention. The above patients can be discharged with assurance to patients.

Introduction

Neonatal Hyperbilirubinemia (NH) is commonest clinical finding in the first week of life. Over two third of newborn babies develop clinical jaundice. The physical finding like yellowish discoloration of the skin and sclera in newborns occurs due to accumulation of unconjugated bilirubin. In most infants, unconjugated hyperbilirubinemia reflects a normal physiological phenomenon.[1] NH affects almost 60% of term and 80% of preterm neonates during first week of life. 6.1% of well term newborn have a serum bilirubin over 12.9 mg%. Serum bilirubin above 15 mg% is found in 3% of normal term newborns. Neonatal Hyperbilirubinemia (NH) has been cause of concern for the parents as well as for the pediatricians.[2]

Early discharge of healthy term newborns after normal vaginal delivery has become a common practice due to medical reasons like prevention of nosocomial infections, economic constraints and also for social reasons like in early naming ceremony. American Academy of Pediatrics also recommends that newborn discharged within first 48 hours of birth should have a follow-up visit after 48 to 72 hours to check for significant jaundice and other problems.[3] Concern of pediatricians regarding the early discharge of such neonates are because of reports of bilirubin induced brain damage occurring in healthy term infants even without hemolysis. The sequalae could be serious as cerebral palsy, sensorineural deafness and mental retardation.[4,5]

There are some simple and easily available markers to predict hyperbilirubinemia in newborns. Some of them are transcutaneous bilirubinometer, umbilical cord blood bilirubin estimation, predischarge hour specific bilirubin estimation and end tidal carbon monoxide measurement.[6] Umbilical cord blood bilirubin estimation is a cheap, readily available and also a non-invasive method which helps in predicting developing jaundice in otherwise healthy newborns, thus aid in decision of early discharge of newborns.[7] Cord blood Bilirubin (CBB)

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2023.4.in178
Vol. 27 Iss. 4 (2023)

concentration measures in-utero hemolysis, thus representing a possible biomarker for severe hyperbilirubinemia secondary to Hemolytic Disease of newborn.[8]

The aim of this study is to predict development of significant neonatal hyperbilirubinemia early in neonatal period in a ABO blood group setting. It also correlates cord blood bilirubin and serum bilirubin at 24hrs and 48 or 72 hours of life and at follow up between 5th to 7th day of life in setting of ABO Incompatibility.

Methods

This was a prospective observational study conducted in NICU of Dhiraj Hospital, Sumandeep Vidyapeeth, Vadodara Gujarat during period of one and half year from March 2021 to September 2022. All healthy inborn Full term babies with A, B or AB blood group born to O positive healthy mothers born during the enrollment period were included in the study.

STUDY TYPE: prospective observational study
SAMPLE SIZE: considering the prevalence of ABO incompatibility cases in last 2 years in NICU of Dhiraj Hospital, Sumandeep Vidyapeeth, sample size for present study was calculated to be 138 using the formula **n=z2p(1-p)/e2**

Exclusion criteria:

1. Rhesus blood factor incompatibility.
2. Blood group O positive babies
3. Major congenital malformations.
4. Chronic maternal illness (like DM, hypothyroidism)
5. Those who didn't give consent for follow up
6. Preterm babies < 37 weeks of gestation.
6. Outborn neonates

ETHICAL CLEARANCE: Ethical Clearance certificate was obtained from Institutional Ethical Committee of Smt. B.K.Shah Medical Institute and Research Centre, Dhiraj Hospital, Sumandeep Vidyapeeth, Pipariya, Waghodiya, Vadodara.

INFORMED CONSENT: All the parents of the enrolled babies were explained about the study and its purpose and informed written consent was taken from them before enrolment.

METHOD OF COLLECTION OF DATA:

- Demographic profile and relevant information was collected by **using structured proforma** by interviewing the mother and relatives.
- Cord blood sample for cord blood bilirubin and blood group is collected from all mothers with O positive blood group in PLAIN vacuttee.
- Then subsequent Serum bilirubin estimation of newborn was sent at 24hrs and 48 or 72 hours of age and on follow up in between 5th to 7th day of life (send SBR ,if neonate is clinically icteric) with ABO incompatibility.
- Daily all babies were clinically assessed for Jaundice and its severity by Kramer's method.[9]

Area of body	**Level of bilirubin**
Face Chest, upper abdomen	4-6 mg/ dl 8-10 mg/dl
Lower abdomen, thighs Arms, lower legs Palms, soles	12-14 mg/dl 15-18 mg/dl 15-20 mg/dl

DOI: 10.5123/inj.2023.4.in178
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Laboratory Investigations

1. Cord blood (2ml) was collected from placental side after its separation and subjected to following investigation.
    a. Blood group
    b. Total and Direct Serum Bilirubin.
2. Venous blood samples were collected from the baby at at 24hrs and 48 or 72 hours of age
    a. Total and Direct Serum Bilirubin.
    b. Retic count, DCT (if required)
    3. Venous blood sample was send on follow up in between 5^{th} to 7^{th} day of life if jaundice is clinically significant.

Serum bilirubin estimation was done by Diazo method. This method for Bilirubin estimation is based on principle that Bilirubin reacts with Diazotised Sulphanilic acid in acidic medium to form pink coloured Azobilirubin with absorbance directly proportional to Bilirubin concentration. Direct Bilirubin, being water soluble directly reacts in acidic medium. However indirect or unconjugated Bilirubin is solubilised using a surfactant and then it reacts similar to direct Bilirubin.

Statistical Analysis

Prospective analysis was carried out in the current study. Quantitative Data were expressed as mean +/- SD (Standard Deviation). Qualitative Data were expressed as percentage. Quantitative variables were measured using Pearson's correlation coefficient. Significance was assessed at 5% level of significance ($p<0.05$) Analysis of Qualitative data was done using Chi-Square test. Diagnostic statistics such as Sensitivity, Specificity, PPV, NPV, Accuracy were also obtained. The statistical analysis of all data was performed using computer program SPSS for Windows and Microsoft excel 2010.

Results

The present clinical study consisted of totally 324 healthy term newborns with MBG O positive and newborn blood group A/B/AB positive, delivered in SBKSMIRC, Dhiraj Hospital, Sumandeep Vidyapeeth during study period.

The prevalence of Jaundice in the study population is 13.5% (n=44).
The Study Population Consisted Of 48.4% Males And 51.6% Females (TABLE 1). 4.6% neonates were weighing between 2.2 to 2.5 kg ,76% neonates weighed between 2.5 to 3 kg 18.2% weighed between 3 to 3.5 kg and 0.6 % above 3.5 kgs. (TABLE 2)

Primigravida were 153 in number (47.3%) and Multigravida were 171 in number(52.7%). (TABLE 3)
13% (44 in number) of the present study population had significant jaundice (TABLE 4). Out of these 44 neonates, 24 were females and 20 were males. Hence, females shows higher incidence of developing significant hyperbilirubinemia than males. Also distribution of significant hyperbilirubinemia amongst the 44 neonates was equal (50% with A + blood group and 50% with B + blood group).

Mean cord bilirubin level to be 2.6 mg/dl (range: 1.9-3.7, SD- $\pm$1.6), the mean total bilirubin at Postnatal 48 TO 72 hours to be 12.13 mg/dl (range:6.5 TO 17.3, SD- $\pm$2.2). (TABLE 5) Also 37 newborns out of 44 who developed significant jaundice had cord blood bilirubin =>3 , 7 newborn out of 44 who developed significant jaundice had cord blood biliriubin <3 (TABLE 6)

Using cord blood bilirubin level > 3 mg/dl, significant hyper bilirubinemia can be predicted with sensitivity of 85%, specificity of 93.5%, negative predictive value of 98% and positive predictive vale of 69%. (TABLE 7)

TABLE NO 1: DISTRIBUTION OF NEONATES ACCORDING TO SEX

GENDER OF NEONATE	NUMBER OF NEONATES	TOTAL PERCENTAGE
MALE	157	48.4%

DOI: 10.5123/inj.2023.4.in178
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

FEMALE	**167**	**51.6%**

TABLE NO 2 : DISTRIBUTION OF NEONATE ACCORDING TO BIRTH WEIGHT

Birth weight in kgs.	Number of neonates	Total %
2.2 TO 2.5KGS	**15**	**4.6%**
2.5 TO 3 KGS	**246**	**76%**
3 TO 3.5 KGS	**59**	**18.2%**
3.5 AND ABOVE	**4**	**1.2%**

TABLE NO 3 : PARITY OF MOTHERS

PARITY	NUMBER OF BABIES	TOTAL PERCENTAGE
PRIMI	153	47.3%
MULTI	171	52.7%
TOTAL	324	100%

TABLE NO 4: STUDY POPULATION AND SIGNIFICANT JAUNDICE

TOTAL PATIENTS	SIGNIFICANT JAUNDICE NUMBER	PERCENTAGE
TOTAL NUMBER 324	44	13%

TABLE NO 5: BILIBUBIN PROFILE OF THE STUDY POPULATION

Parameters	Minimum	Maximum	Mean	SD (±)
Cord Blood Total Bilirubin (mg/dl)	1.9	3.7	2.60	1.6
Postnatal 24hours Bilirubin (mg/dl)	3.5	12.4	7.99	1.7
Postnatal 48 72hours Bilirubin (mg/dl)	6.5	17.3	12.13	2.2

TABLE 6 : DIAGNOSTIC PREDICTABILITY OF CORD BLOOD BILIRUBIN FOR SIGNIFICANT JAUNDICE

DOI: 10.5123/inj.2023.4.in178
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Cord bilirubin	Significant jaundice	Neonates without jaundice
>=3	37	18
<3	7	266

TABLE NO 7 : PREDICTABILITY OF CORD BLOOD BILIRUBIN FOR HYPERBILIRUBINEMIA

True positive	37
False positive	18
False negative	7
True negative	262
Sensitivity	85%
Specificity	93.5%
PPV	69%
NPV	98%

Discussion:

ABO incompatibility, usually a problem of neonate rather than fetus, is one of the most common materno-fetal blood group incompatibility, resulting most commonly in Jaundice in 1st 24 hours of life. Our study hypothesis was high peak of Serum Bilirubin later in 1st-2nd week of life can be predicted by high levels of Bilirubin at birth. Hence, cord blood bilirubin investigation was chosen as it is non-invasive, results are obtained in short duration and can be useful in resource limited rural settings also.

In the present study, study group is uniformly distributed with male 157and female 167 babies. There is no significant correlation (p 0.89) in the TSB levels and the sex of the newborn. This results correlate with the results of Dufour DR et al, where it shows no correlation of sex, race, gravidity, birth weight, and blood type of neonate with neonatal hyperbilirubinemia.[10]but studies like Maisel et al, show male sex in the study group is 74.8% compared to control with 49.6%, with p value 0.007.[11]

Incidence of significant hyperbilirubinaemia was 13 %. Several studies shows this range varying from 8.3% to 12.5%.[12,13,14] Also the distribution was equal amongst both the sex of babies. However in Bhutani et al, more significant jaundice was seen in O-A blood group than O-B or O-AB blood group incompatibilities.[6] The previous literatures have also mentioned inconsistencies with regard to the degree of hemoysis and incidence and severity of hyperbilirubinaemia amongst O-A or O-B pairs. Kaplan et al has showed more significance and severity with O-B group using ETCO2 as predictor of hyperbilirubinemia.[15]

>=3 mg/dl CBB was used as a cut-off value for predicting significant hyperbilirubinaemia in the present study. Many studies have researched and reported correlation between high levels of cord blood bilirubin with the prediction and development of significant hyperbilirubinaemia. But few studies like Azma Et al done in 2011, have reported no correlation amongst such variables.[16]

Using >= 3 mg/dl as cut off in our study, hyperbilirubinaemia can be predicted with sensitivity of 85%, specificity of 93.5%, Positive predictive value of 69% and Negative predictive value of 98%. The correlation between cord bilirubin and development of significant Jaundice in the postnatal 48 to 72 hours showed a positive correlation according to Karl Pearson's correlation coefficient method.(r = 0.37).

In a study by Pradhan et al, using CBB of 2.5 mg/dl, sensitivity was 84.1%, specificity was 88.5% NPV of 45% and PPV of 98% for development of significant Jaundice.[17] whilst study by Calkins et al, established CBB cut off of 2.05 mg/dl has 80% sensitivity and 78% specificity for prediciting administration of phototherapy.[8] The study done in 2018 by Janaki et al has sensitivity of 70.6 % and specificity of 82.7% using CBB cut off of 1.85 mg/dl.[18] Farhat et al in 2013, had PPV of 66%, NPV of 64.2%, sensitivity of 68.8% and specificity of 61.8% with CBB cut off value of 2 mg/dl.[19]

However, study had certain limitations as it has included only term newborns and sample size was comparatively small.

Conclusions

From the above results and discussion, umbilical cord blood bilirubin was found to be important indicator in prediction of significant jaundice in 1st week of life. A 98% Negative Predictive Value in the present study suggests that in healthy Term babies with ABO without Rh incompatibility Cord Blood Bilirubin <3 mg/dl can help to identify those newborns who are unlikely to require further evaluation and intervention. Babies with Cord Blood Bilirubin level ≥ 3 mg/dl should be followed more frequently to reduce mortality and morbidity due to Neonatal hyperbilirubinemia.

References

1. Meharban Singh. Care of the Newborn. 7th ed. New Delhi: Sagar Publications; 2010. Chapter 18, Neonatal Jaundice, p 254-74.
2. Radmacher P, Massey C, Adamkin D. Hidden Morbidity With "Successful"
3. Early Discharge. J Perinatol. 2002;22:15-20.
4. American Academy Of Pediatrics, Clinical Practice Guideline; Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation,
5. Pediatrics 2004;114(1):297-316
6. Penn AA, et al. Kernicterus in a full term infant. Pediatrics 1994;93:1003-1006.
7. Maisels MJ, et al. Kernicterus in otherwise healthy breast-fed term newborns, Pediatrics 1995;96:730-733.
8. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of predischarge hour specific serum bilirubin for significant hyperbilirubinaemia in healthy term and near-term newborns. Pediatrics. 1999; 103(1):6-14.
9. Kardum D, Biljan B, Santic K, Kos M. Cord blood bilirubin and prediction of NHB and perinatal infection in newborns at risk of hemolysis J Pediatr (Rio J). 2021;97(4):440-4.
10. Calkins K, Roy D, Molchan L, Bradley L, Grogan T, Elaschoff D, et al. Predictive value of cord blood bilirubin for hyperbilirubinemia in neonates at riskr maternal-fetal blood group incompatibility and haemolytic disease of newborn. J Neonatal Perinatal Med. 2015;8(3):243-50
11. Kramer LI. Advancement of dermal icterus in the jaundiced newborn. Am J Dis
12. Child 1969 ; 118 : 454-458.
13. Dufour DR, Monoghan WP. ABO haemolytic diseases of newborn. A retrospective analysis of 254 newborns. Am J Clin Pathol. 1980;73(3):369-73
14. Maisles MJ, Mcdonag AF. Phototheraphy for Neonatal Jaundice. N Engl J Med. 2008 Feb 28;358:920-8.
15. Agarwal R, Deorari AK. Unconjucated Hyperbilirubinemia in Newborn. Indian Pediatr. 2002 Aug 17;39:30-42.
16. Knupfer M, Pulzer F, Gebauer C, Robel-Tillig E, Vogtmann C. Predictive value of umbilical cord blood bilirubin for postnatal hyperbilirubinaemia. Acta Paediatr. 2005 May;94(5):581-7.
17. Randew S, Grower N. Predicting neonatal hyperbilirubinemia using first day serum bilirubin levels. Indian J Pediatr. 2010 Feb;77:147-50.
18. Kaplan M, Hammerman C. Understanding severe hyperbilirubinaemia and preventing Kernicterus: adjuncts in interpretationof neonatal serum bilirubin. Clin Chim Acta. 2005,356(1-2):9-21
19. Azma RZ, Farshya SMZ, Nurasykin Y, Noriya M, Nabeelah AK. Prediction of significant neonatal jaundice in babies with ABO incompatibility by Cord blood tests. Med Health2011;6:292-3
20. Pradhan A, Lamhaney R, Sharma V. Cord blood bilirubin level as a predictor of development of pathological hyperbilirubinemia in newborns. Int J Contemp Pediatr. 20174(4):;1519-24
21. Janaki AN, Selvakumar P. Predicitve valueof umbilical cord blood bilirubin and albumin for significant hyperbilirubinaemia in ABO incompatibility. Int J Pediatr Res. 2018;5(1):24-30
22. Farhat A, Ghamsari AA, et al. Does umbilical cord bilirubin level predictive value in pathologic NHB? Iranian J Neonatal. 2013;4(1):32-5