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# CORD BLOOD BILIRUBIN AND ALBUMIN AS PREDICTORS OF NEONATAL HYPERBILIRUBINEMIA IN HEALTHY TERM BABIES

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**ABSTRACT**

**Background & Objectives:** Neonatal jaundice is the most common problem encountered in newborns and is universal. Development of hyperbilirubinemia in neonates is fretful for the parents and a concern for the pediatrician too.To assess the predictive value of cord blood bilirubin and albumin in identifying healthy term babies at risk of developing hyperbilirubinemia; TO establish cut off values of cord blood Bilirubin and Albumin to identify healthy term newborn babies at risk of developing hyperbilirubinemia; To assess the prevalence of Significantneonatal hyperbilirubinemia in healthy term babies.

**Methods:**A prospective study was carried out in the Department of biochemistry, Andhra medical college, King George Hospital Visakhapatnam from September 2020 to August 2021with prior approval from Instituitional Scientific and Ethics Committee. A pre designated Proforma has aided the enrolment of the newborns into the study.

**Results:**The present study included 60 term healthy newborn babies born at King George Hospital, Visakhapatnam during the period September 2020 to August 2021.The mean cord blood bilirubin level is 1.8 with a range of 0.2 to 3.1 and the mean cord blood albumin level is 3.1 with a range of 2.1 to 4.1.

**Conclusion:**Cord blood bilirubin > 1.85 mg/dl, cord blood albumin < 3.05 mg/dl and cord blood bilirubin albumin ratio > 0.59 were found to have good predictive value in identifying newborns who are likely to develop neonatal hyperbilirubinemia.Newborns who are at higher risk of developing neonatal hyperbilirubinemia can be identified using the above criteria and thus can be followed up and treated vigorously to avoid dangerous complications like Kernicterus.New borns who are at low risk of developing hyperbilirubinemia can be identified as well and can be discharged early, thus avoiding unnecessary economic and psycho-social burden on the parents and the risk of hospital acquired infection in the babies

**Keywords:** Jaundice, cord blood, albumin,Premature, New born.

## INTRODUCTION

Neonatal jaundice is the most common problem encountered in newborns and is universal. Jaundice is seen in 60-70% of term babies and 80% of preterm babies [1,2] in the 1st week of life. This is due to immature liver cells having very low uridine diphosphate glucuronyl transferase activity compared to mature hepatocyte and higher volume of shortened life erythrocytes in circulation. This is called physiological jaundice. However non physiological or pathological hyperbilirubinemia is known to occur in about 6-10% of healthy term newborns [3,4,5]. About 6.1% of jaundiced neonates have maximum bilirubin over 12.9mg/dl & 3% term babies have high serum bilirubin levels > 15mg/dl[6] .In about 6.5 percent of babies, hyperbilirubinemia is the most common cause for re-admission during early neonatal period [7]. 85 percent of term newborns who are readmitted during their first week of life are admitted for neonatal jaundice [8].

The role of Bilirubin in the newborn has been an enigma because of its dual role as a potent natural antioxidant and at the same time, a cytotoxic agent producing brain dysfunction. Since there is no cut off value of the bilirubin level that can cause bilirubin encephalopathy, neonatal jaundice is a serious concern for both parents and pediatricians [9]. Severe Jaundice and bilirubin encephalopathy can occur in some full term healthy newborns with no apparent clinical findings of hemolysis, who are discharged early [10] . It is very difficult to know who are at increased risk for developing significant hyperbilirubinemia (Total serum Bilirubin >= 15mg/dl) [9]. The concept of early predicting jaundice early using cord blood bilirubin has been studied, but the results were not consistent [11,12,13,14]. Various methods have been suggested for early detection of neonatal jaundice such as physical examination, use of risk factor table, routine pre discharge transcutaneous bilirubin measurement and also by measurement of expiratory carbon monoxide levels.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE8, 2023

Albumin is synthesized by liver and helps in transport of unconjugated bilirubin.

**AIMS AND OBJECTIVES**

**AIM**: To Estimate cord blood Bilirubin and Albumin levels in healthy term babies born in obstetrics and gynecology department at King George Hospital, Visakhapatnam.

**OBJECTIVES:**

1) To assess the predictive value of cord blood bilirubin and albumin in identifying healthy term babies at risk of developing hyperbilirubinemia
2) To establish cut off values of cord blood Bilirubin and Albumin to identify healthy term newborn babies at risk of developing hyperbilirubinemia
3) To assess the prevalence of significant neonatal hyperbilirubinemia in healthy term babies.

**MATERIALS AND METHODS**

A prospective study was carried out in the department of biochemistry, Andhra medical college, King George Hospital Visakhapatnam from September 2020 to August 2021 with prior approval from Instituitional Scientific and Ethics Committee. A pre designated Proforma has aided the enrolment of the newborns into the study.

**INCLUSION CRITERIA**

- Term baby(>_37weeks)
- Birth weight(>_2.5 kgs)
- APGAR>_7 at 5 mins

**EXCLUSION CRITERION**

>Preterm baby (<37 weeks) and post term (>_42 weeks)
>Rh incompatibility
> ABO incompatibility
>Congenital anomalies
>APGAR < 7 at 5 min
Sick neonates

Those whose mothers are not willing to give consent

**METHODOLOGY**

Cord blood of about 2ml was collected under strict aseptic precautions soon after the delivery from the placental side after its separation. The blood collected is stored away from light and the sample is refrigerated between 2-8 degrees centigrade till bilirubin estimation is done. Blood grouping and typing were also done on cord blood.

Bilirubin estimation is done by JENDRASSIK GROF method which measures Direct and Total bilirubin levels (Indirect bilirubin levels are calculated). This method is based on fact that bilirubin reacts with diazotized sulfanilic acid in acidic medium to form azobilirubin, a pink colored complex whose absorbance is proportional to Bilirubin concentration. Direct bilirubin being water soluble is allowed to react with diazotized sulfanilic acid in the absence of an activator while for total bilirubin the diazotization is carried out in the presence of an activator.

Cord blood Albumin levels were estimated by BROMOCRESOL GREEN (DYE BINDING) method in auto analyzer based on the principle that Albumin in buffered medium binds with bromocresol green and produces a green colored complex whose absorbance is proportional to Albumin concentration.

Neonates were followed up daily for 5 days to look for any significant jaundice by clinical assessment using Kramer‟s rule and bilirubin estimations were done in those who developed significant jaundice as and when required. A detailed case proforma was prepared.

**TOTAL BILIRUBIN**

Jendrassik and Grof method

A stabilized diazonium salt, 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacts with bilirubin to form azobilirubin which absorbs at 570/660 nm. Caffeine and serum interfernces.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE8, 2023

Bilirubin + DPD → caffeine & surfactant → Azobilirubin

**Reagent Composition:**

Caffeine: 2.1 m mol/L

3,5- Dichlorophenyldiazonium Tetrafluoroborate: 0.31 mmol/L Also contains preservative and surfactant.

**Sample collection and preparation:**
Serum or heparinized plasma, free from hemolysis is the recommended specimen. Sample should be protected from light. Exposure to direct sunlight can decrease bilirubin in samples by 50% within one hour. Upon well protection from light, bilirubin in serum is stable for 3 days when stored at 2-8$^0$ C, or three months when stored at $\leq$ -20$^0$ C.

**DIRECT BILIRUBIN**
Modified Jendrassik Method

**Principle:**
Direct (conjugated) bilirubin couples directly with a diazonium salt of 3,5- dichloroaniline (DPD) in an acid medium to form azobilirubin.
The direct bilirubin in serum is directly proportional to the color development of azobilirubin which is measured bichromatically at 570/660 nm.
Bilirubin + 3,5-Dichlorophenyl Diazonium → Azobilirubin.

**Expected Values Adults:** 0.03-0.18 mg/dl

**ALBUMIN**
Bromocresol green dye binding method

**Principle:** This method is based on specific binding of Bromocresol green (BCG), an anionic dye, with albumin in a buffered medium to produce a green colored complex. The intensity of color formed is proportional to the concentration of albumin in the sample measured at 630 nm
BCG + Albumin PH 4.3 BCG – Albumin complex (green colored complex)

**ETHICAL ISSUES**
A special informed consent was taken from all the mothers before including their baby in the study. No special consent was required for collection of venous blood for bilirubin if the baby under study developed significant jaundice, as it is a part of patient care.

**STATISTICAL ANALYSIS**
Data was analyzed using a computer aided statistical package SPSS. The statistical methods used were ANOVA, chi-square test, odds ratio, multiple logistic forward step wise (conditional method), multiple logistic regression likelihood ratio, students test and ROC curves (receiver operating characteristic curves). The cut off value for cord bilirubin and albumin for predicting significant hyperbilirubinemia was established using ROC curves. ROC curves were plotted using 1-specificity on the X axis and sensitivity on Y axis.

**RESULTS**
The present study included 60 term healthy newborn babies born at King George Hospital, Visakhapatnam during the period September 2020 to August 2021.The mean cord blood bilirubin level is 1.8 with a range of 0.2 to 3.1 and the mean cord blood albumin level is 3.1 with a range of 2.1 to 4.1

**TABLE 1: GROUP STATISTICS SHOWING DEMOGRAPHIC PARAMETERS OF STUDY POPULATION**

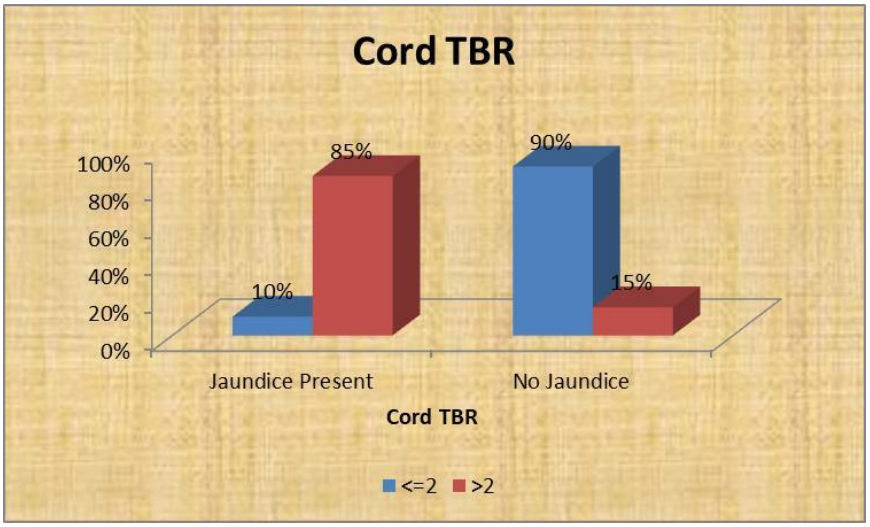
| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Age of mother | 60 | 18.00 | 40.00 | 24.7167 | 4.68351 |
| BW | 60 | 2.40 | 4.20 | 2.9173 | .33818 |
| Cord TBR | 60 | .20 | 3.10 | 1.8267 | .44450 |
| Cord DB | 60 | 0.00 | .60 | .3150 | .13756 |
| Cord IBR | 60 | .20 | 2.70 | 1.5117 | .37647 |
| Cord ALB | 60 | 2.10 | 4.10 | 3.1133 | .42684 |

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE8, 2023

**TABLE NO 2: CORD BLOOD BILIRUBIN VS JAUNDICE**

| TABLE NO:2 | | | Jaundice | | Total |
|---|---|---|---|---|---|
| | | | Present | Nil | |
| Cord TBR | <= 2 mg/DL | Count | 5 | 37 | 42 |
| | | % | 11.9% | 88.1% | 100.0% |
| | >2.1 mg/DL | Count | 16 | 2 | 18 |
| | | % | 88.9% | 11.1% | 100.0% |
| Total | | Count | 21 | 39 | 60 |
| | | % | 35.0% | 65.0% | 100.0% |
| Chi-Square Value = 32.824 | | | | | |
| P Value = 0.000 | | | | | |

**GRAPH 1: CORD BLOOD TOTAL BILIRUBIN VS JAUNDICE**




**In babies with cord total bilirubin more than 2mg/dl, 85% developed jaundice where CORD BLOOD ALBUMIN ITS IMPACT ON NEONATAL JAUNDICE**

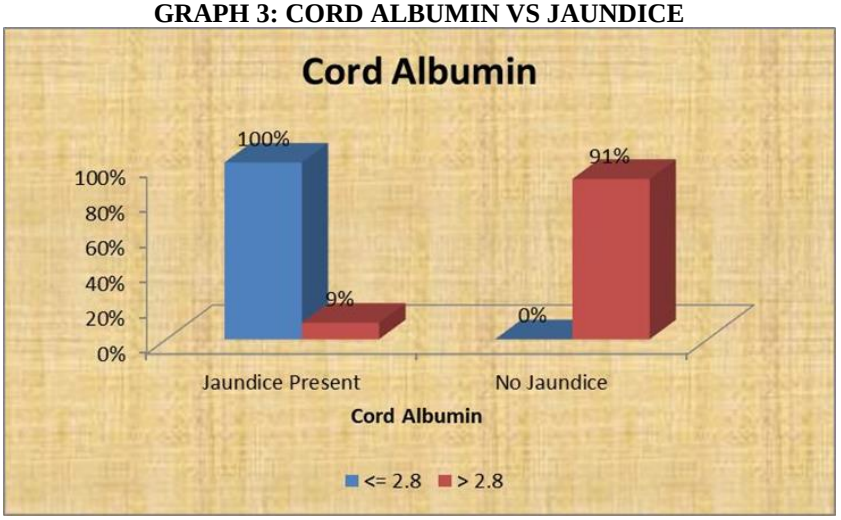
Out of 21 babies who developed jaundice, 17 were found to have cord blood albumin values less than or equal to 2.8 mg/dl and only 4 were found to have cord blood albumin values more than 2.8 mg/dl.

**TABLE NO 3: CORD BLOOD ALBUMIN VS JAUNDICE**

| | | | Jaundice | | Total |
|---|---|---|---|---|---|
| | | | Present | Nil | |
| Cord Alb Cat | <= 2.8 | Count | 17 | 0 | 17 |
| | | % | 100.0% | 0.0% | 100.0% |
| | > 2.8 | Count | 4 | 39 | 43 |
| | | % | 9.3% | 90.7% | 100.0% |
| Total | | Count | 21 | 39 | 60 |
| | | % | 35.0% | 65.0% | 100.0% |
| Chi-Square Value = 44.053 | | | | | |
| P Value = 0.0002 | | | | | |

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE8, 2023

**GRAPH 3: CORD ALBUMIN VS JAUNDICE**




In babies with cord blood albumin less than or equal to 2.8mg/dl all (100%) of them developed jaundice where as in babies with cord blood albumin more than 2.8mg/dl only 9% of them developed jaundice

**DISCUSSION**

Serum bilirubin levels are usually 1-3mg/dl at birth and rise at the rate of less than 5mg/dl per day, peaking at 2-3 days in term neonates. Our study hypothesis was that an increased serum bilirubin level at birth would also predict a high peak later in newborn period. Our aim is to study the relationship between cord blood bilirubin and albumin with serum bilirubin levels in jaundiced babies and there by establish cut off values which will help in early prediction of neonatal jaundice. We choose cord blood estimation for initial serum bilirubin estimation because it is a non-invasive way and the results are available within few hours after birth. We have taken serum bilirubin levels more than 12mg/dl as hyperbilirubinemia and serum bilirubin more than 15mg/dl as significant hyperbilirubinemia since specific treatment is considered at or above this level.

The growing practice of early discharge of newborns owing to common medical, social and economic reasons resulted in re-emergence of bilirubin related neurological sequelae. It has been shown that newborns discharged within 72 hours of delivery are at significantly greater risk for readmission than those whose stay is more than 72 hours [66,67,68,69] and Hyperbilirubinemia is the most common cause for readmission [62,66,68,69].Further 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 [70,71].

Therefore it is crucial to catalogue the babies who are at risk for developing jaundice by establishing safe markers. Development of safe markers will help in preventing fatal outcome due to jaundice. The desperate need for early prediction of jaundice and paucity of studies from India has spurred us to undertake this prospective follow-up study.

Our study included 60 term healthy neonates born at King George Hospital Visakhapatnam, a teritiary care hospital. The incidence of significant hyperbilirubinemia in our study was found to be 13% (8 out of 60) which is in correlation with other studies.

**INCIDENCE OF HYPERBILIRUBINEMIA**

**TABLE 4: COMPARISON OF INCIDENCE OF HYPERBILIRUBINEMIA**

| Studies | Year | No. Of Cases | Incidence of significant Hyperbilirubinemia % |
|---|---|---|---|
| Palmer *et al*[52] | 1983 | 41057 | 10.70 |
| Phurpradit *et al*[56] | 1993 | 7644 | 8.35 |
| Awasthi *et al*[62] | 1998 | 274 | 12.80 |
| Alpay *et al*[63] | 2000 | 498 | 12.05 |
| Agarwal *et al*[64] | 2002 | 213 | 10.30 |
| KnupferM *et al*[60] | 2005 | 1100 | 10.60 |
| RandevS *et al*[65] | 2010 | 200 | 12 |
| Present Study | 2021 | 60 | 13 |

The present study is in correlation with studies conducted by Awasthi *et al*, Alpay *et al* and Knupfer M *et al*.With regard to association of **cord blood bilirubin** and jaundice, in the present study, ROC curve for cord bilirubin established cut

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off value of **≥1.85mg/dl** which had sensitivity of 85%, specificity of 90%, positive predictive value of 82.07% and negative predictive value of 91.76%.

**TABLE 5: Cord blood bilirubin cut off values determined by various studies**

| Name of study | Cordblood bilirubin cut off | sensitivity | specificity | NPV |
|---|---|---|---|---|
| Knudsen *et al* | 2.33mg/dl | 13% | 99% | 72% |
| Knupfer *et al* | 1.74mg/dl | 97% | 41.4% | 99.8% |
| Amar taskande *et al* | 2 mg/dl | 89.5% | 85% | 98.7% |
| Zakia Nahar *et al* | 2.5mg/dl | 77% | 98.6% | 96% |
| Singhal V *et al* | 1.9mg/dl | 90% | 82.5% | 98.07% |
| Our study | 1.85mg/dl | 85% | 90% | 91.76% |

All the studies showed significant association between umbilical cord bilirubin and development of jaundice in subsequent post natal days. The present study shows that cord blood bilirubin level can be used as an useful predictor of subsequent neonatal hyperbilirubinemia and will help in identifying the low risk group children with cord blood bilirubin level <1.85mg/dl. As the negative predictive value for this cut off is 91.76%, it will be an important parameter while taking a decision about discharging babies early.

Albumin helps in hepatic transportation of bilirubin and its clearance. Low serum albumin levels decrease bilirubin clearance and thus increases significant hyperbilirubinemia. The ability of cord blood albumin to act as a tool for predicting neonatal jaundice was assessed in the current study. There was a significant higher cord serum albumin level in neonates who did not develop neonatal hyperbilirubinemia in comparision to those who did. 80.9% (17 out of 21) of cases that developed jaundice had cord albumin levels less than or equal to 2.8gm/dl. In this study cord serum B/A ratio proved to predict the development of significant neonatal hyperbilirubinemia. ROC curve for cord bilirubin albumin ratio (B/A) established cut off value of 0.59 which had sensitivity of 95.20%, specificity of 97.40%, positive predictive value of 91.10% and negative predictive value of 97.24%. The p value is 0.00008 which is significant.

From our study it is evident that Cord Albumin ≤ 3.05 gm/dl is a better predictor followed by Cord bilirubin albumin ratio of ≥ 0.59 and cord total bilirubin of ≥ 1.85 mg/dl.
The present study is in correlation with studies conducted by Knudsen *et al*, Amar Taksande *et al*, Rostami *et al* where no significant association could be demonstrated between mode of delivery and the incidence of neonatal jaundice.

Considering the impact of parityon incidence of jaundice, the present study showed that 10 out of 29 neonates (34.5%) born to primi mothers and 11 out of 31 neonates (35.5%) born to multiparous mothers developed jaundice. This shows there is no significant association between parity of mother and neonatal jaundice.

Study done by Hamidi *et al* (2012) showed no significant association between parity of mother and neonatal jaundice. our study is in correlation with study conducted by Hamidi *et al*.

**LIMITATIONS OF THE STUDY**
Only full term neonates were included in this study so the study results may not be applicable to preterm neonates.Neonates were only followed up to Day 5 of life. So certain late onset neonatal jaundice cases might have been missed. But such cases are very rare.Neonates were evaluated clinically for the presence of jaundice and clinical assessment might not be considered very accurate by some.

**CONCLUSION**
Cord blood bilirubin > 1.85 mg/dl, cord blood albumin < 3.05 mg/dl and cord blood bilirubin albumin ratio > 0.59 were found to have good predictive value in identifying newborns who are likely to develop neonatal hyperbilirubinemia.Newborns who are at higher risk of developing neonatal hyperbilirubinemia can be identified using the above criteria and thus can be followed up and treated vigorously to avoid dangerous complications like Kernicterus.New borns who are at low risk of developing hyperbilirubinemia can be identified as well and can be discharged early, thus avoiding unnecessary economic and psycho-social burden on the parents and the risk of hospital acquired infection in the babies.

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**CONFLICT OF INTEREST** None

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