**ORIGINAL RESEACH ARTICLE**

# Diagnostic Utility of Cord Blood Bilirubin in Early Detection of Neonatal Hyperbilirubinemia among ABO Incompatibility Cases from a Tertiary Care Medical College Hospital.

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***Abstract:***

***Background: The most common medical condition requiring immediate intensive care and repeated hospitalization and or re-admissions in early neonatal period is Neonatal hyperbilirubinemia, which sets in during the first week of newborn's life, where the levelof unconjugated bilirubin in serum increases morethan 1.8mg/dl (Unconjugated hyperbilirubinemia). Purpose: To determine and evaluate the correlation of Cord Blood Bilirubin levels and 4th day serum bilirubin for early prediction of Pathological Neonatal Hyperbilirubinemia in neonates with risk of ABO incompatibility. Methods: The study was conducted as a Prospective epidemiology oriented clinical study in the Department of Pediatrics of our hospital. A total of 135 neonates were included in the study. Results: Among term neonates born to mothers with $O^+$ blood group, nearly 83% (111 out of 135) of the venous cord blood samples had measurable hyperbilirubinemia. Among them, clinical jaundice (Physiological) formed the major portion in98 neonates (73%) while the rest 13 neonates (10%) had pathological jaundice. The mean gestational age of the study population was 38.68 ± 1.37 weeks. The incidence of physiological jaundice and pathological jaundice was high i.e., 60.20% and 61.53% respectively in 2.5-3kg birth weight category. The specificity and sensitivity for a cut-off of ≥ 3mg/dL was calculated for umbilical cord blood bilirubin and was found to be 93% and 97.6% respectively. Similarly the Positive Predictive Value (PPV) was calculated to be 84.6% and Negative Predictive Value (NPV) was 98.4%. Conclusion: Cord blood bilirubin assay (CBBA) is an easy, feasible, non-invasive, cost-effective, time saving, early predictive marker to diagnose the development of pathological jaundice (Unconjugated hyperbilirubinemia) in a setting of ABO incompatibility at birth. A cord blood bilirubin at birth of ≥2.3mg/dl should raise a suspicion of evolving pathological hyperbilirubinemia in neonates. CBBA could be used as a reliable early marker for neonatal hyperbilirubinemia especially in developing and underdeveloped countries of the world.***

***Key words: Cord Blood Bilirubin, Fourth day bilirubin, Jaundice, Newborn, Predictive marker, Sepsis.***

## Introduction:

The most common medical condition requiring immediate intensive care and repeated hospitalization and re-admissions in early neonatal period is Neonatal hyperbilirubinemia, which sets in during the first week of newborn's life, The most common medical condition requiring immediate intensive care and repeated hospitalization and re-admissions in early neonatal period is Neonatal hyperbilirubinemia.

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which sets in during the first week of newborn's life, where the level of unconjugated bilirubin in serum increases more than 1.8mg/dl ie., Unconjugated Hyperbilirubinemia[1-3]. Neonatal hyperbilirubinemia (NNH) is observed approximately in 60% of the Term and 80% of Preterm infants [4]. An elevated serum bilirubin level of >15mg/dl is found in approximately 3% of normal term babies[5]. This results in significant morbidity, increased duration of hospital stay thereby exposing naive neonate to the infectious hospital environment, further leading to excess expenditure for the family, mental agony to the mother and family, risk of decreased breast feeding and finally results is early weaning of the baby[6]. The incidence of Rh- incompatibility and its induced complications like Bilirubin Encephalopathy, Cerebral Palsy, Sensori-Neural Hearing Loss and Mental Retardation etc., were reduced by adhering to effective preventive and treatment guidelines. Feto-maternal ABO incompatibility causing neonatal jaundice exists in about 20-25% of all pregnancies but hemolytic disease of new born sets in only 10% of those cases[7, 8]. Recently ABO has emerged as the common cause of NNH as the probability of developing unconjugated hyperbilirubinemia in these new borns within 3 days (72 hours) of life was about 2.6 times higher[9] and this is attributed to the common institutional practice to discharge both the mother and the new born early especially in developing countries like India. Infants discharged within 48 hours of life show an increasing trend of total serum bilirubin (TSB) and some of them may develop significant jaundice requiring treatment. Feasible, effective and reliable diagnostic strategies followed by prompt theraputic management would ultimately reduce the burden of Neonatal hyperbilirubinemia. Though the routinely used gold standard diagnostic test to detect pathological jaundice is Fourth day serum bilirubin assay; in this study we had validated the estimation of Cord Blood Bilirubin level as a early prediction marker to diagnose and manage and further prevent the adverse complications of NNH in hospital settings. As not many study reports about correlation of Cord blood bilirubin and 4th day bilirubin were found in this region, we took up this study

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## Material & Methods:

The study was conducted as a Prospective epidemiology oriented clinical study in the Department of Pediatrics, Karpagam Medical College Hospital affiliated to Karpagam Faculty of Medical Sciences and Research (KFMS&R), Othakalmandapam, Coimbatore, Tamil Nadu, India, between July 1, 2014 and December 31, 2015 for a period of 18 months. Institutional Human Ethics Committee clearance and parent's informed written consent were obtained prior to the commencement of this research study.

A total of 135 cases of Neonates with A, B or AB blood group born to mothers with O positive blood group were included by random selection as study population and were followed up clinically and by laboratory investigation during the first week of life. Three milliliters of umbilical cord blood was collected with a sterile syringe in the delivery room (Labor ward or Operation Theatre) and was sent immediately (within 30 minutes) to Biochemistry and Microbiology Laboratory, CSL, Karpagam Medical College Hospital for the following work-up: Cord blood bilirubin levels (Conjugated, Unconjugated and total serum bilirubin) by Colorimetric Diazo Method, TSH levels and Blood grouping and Rh typing were done at birth. Serum was collected on the $4^{th}$ day of birth from the neonates managed in Neonatal Intensive Care Unit (NICU) and was sent to the Biochemistry Laboratory for detection of $4^{th}$ day serum bilirubin levels. Cord blood was also sent to Diagnostic Microbiology Laboratory for performing Automated blood culture by Bac'T'Alert system and also to Serology section to rule out presence of HBV, HCV and Dengue infection by Rapid Immuno-chromatographic Card Tests.

**Outcome Measures :** Pathological hyperbilirubinemia or Pathological Jaundice is defined as serum total bilirubin level > 15mg/dl on $4^{th}$ day of life or any total serum bilirubin more than the $95^{th}$ centile for the age in hours taken. The main outcome was analyzed in terms of hyperbilirubinemia requiring phototherapy as per AAP guidelines.

**Data Analysis:** Statistical Data Analysis was done using IBM SPSS version 20.0 software

**Inclusion criteria:**

1. Newborn with A or B or AB blood group born to $O^+$ mothers.
2. Newborn with Gestational Age (GA) >37 weeks
3. Newborn with birth weight 2.5-4kg
4. Newborn with APGAR score > 7.

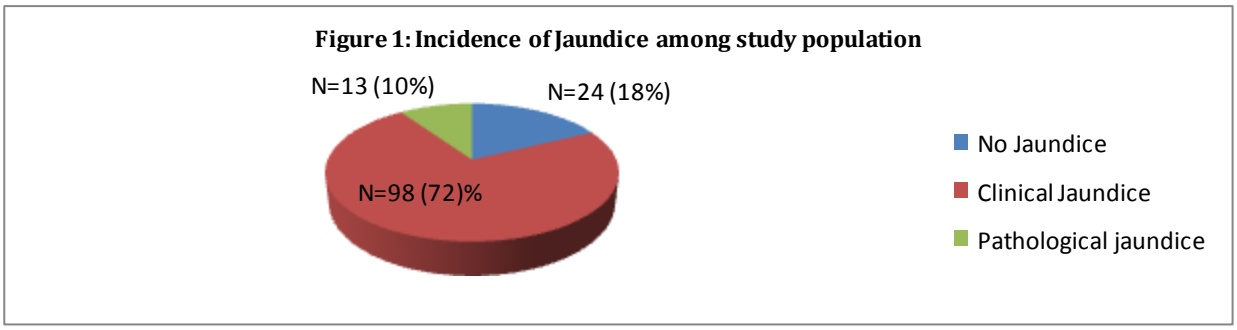
**Exclusion Criteria:**

1. Neonatal problems causing hyperbilirubinemia like Birth asphyxia, Sepsis, Trauma conditions like Cephalhematoma, Hypothyroidism, Congenital malformation, Respiratory distress Syndrome.
2. Neonates born to mothers with Hepatitis B virus (HBV) infection, Hepatitis C Virus (HCV) infection, Dengue fever, Sepsis, Gestation Diabetes Mellitus and Pregnancy Induced Hypertension. Newborn with Rh-incompatability.

## Results:

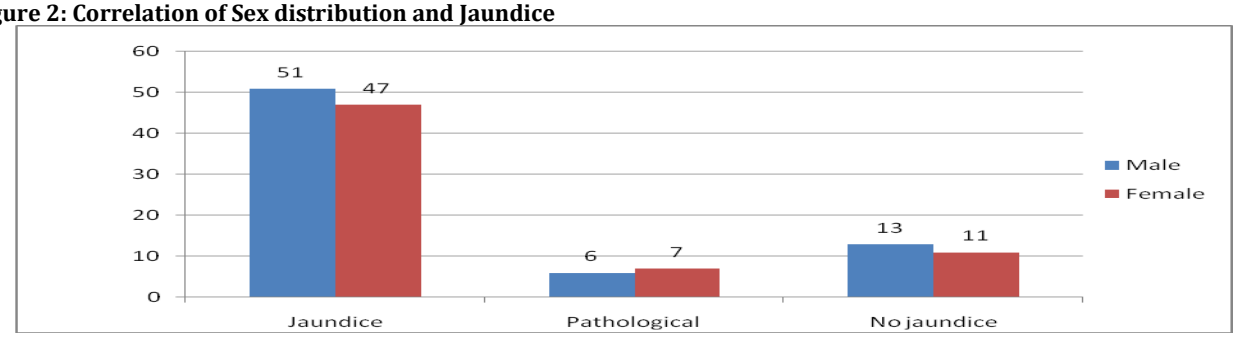
Out of total 135 term neonates born to mothers with $O^+$ blood group, nearly 111 out of 135 (83%) of the venous cord blood samples had elevated bilirubin levels.

**Figure 1: Incidence of Jaundice among study population**




Among them, 98 out of 135 (73%) had clinical jaundice (Physiological) while the rest 13 out of 135 (About 10%) had pathological jaundice. The remaining 17% (24/135) of the total neonates had normal bilirubin levels as depicted in Figure 1.

**Figure 2: Correlation of Sex distribution and Jaundice**




Out of the 135 neonates 72 (53%) of the babies were males and 63 (47%) were females. The mean gestational age of the study population was 38.68 ± 1.37 weeks. The incidence of clinical and pathological hyperbilirubinemia was not statistically significant (p=0.92) in both sexes. Clinical jaundice was apparent in 51 out of 98 (52%) males and 47 out of 98 (48%) female neonates. Out of 13 neonates with pathological jaundice 6 (46.15%) were found to be males and 7 (53.85%) were females as seen in Figure 2.

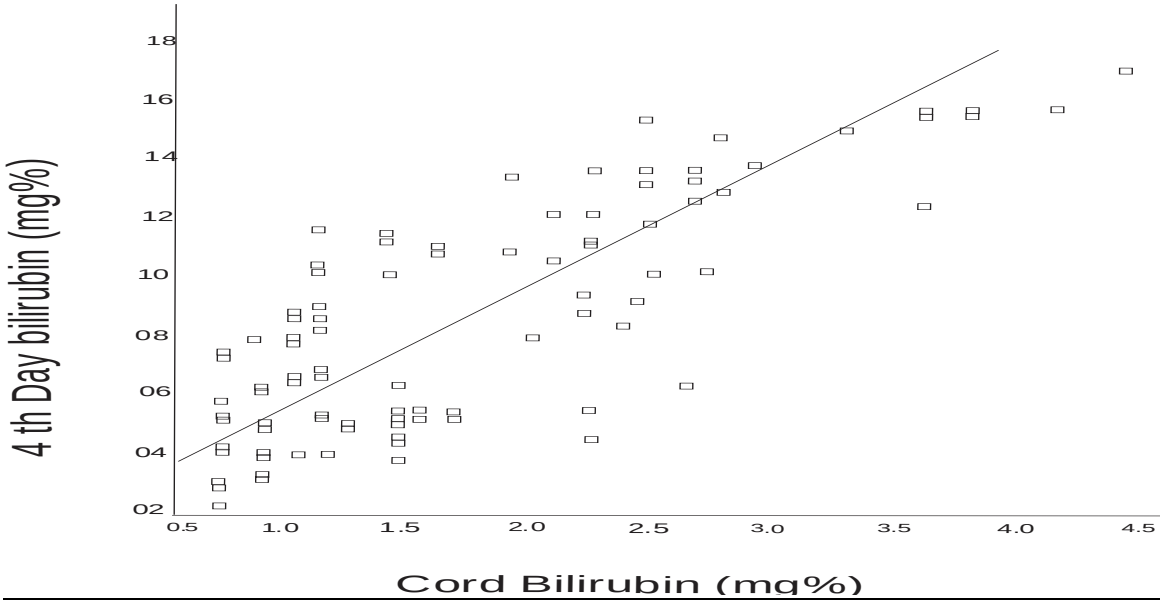
| Table 1: Baby's birth weight vs Incidence of Neonatal Hyperbilirubinemia | | | |
|---|---|---|---|
| **Birth weight** | **No Jaundice** | **Clinical Jaundice** | **Pathological Jaundice** |
| **2.5-3.0 Kg** | **12 (50.00%)** | **59 (60.20%)** | **8 (61.53%)** |
| **3.0-3.5 Kg** | **10 (41.67%)** | **35 (35.71%)** | **4 (30.77%)** |
| **3.5-4.0 Kg** | **2 (8.33%)** | **4 (4.08%)** | **1 (7.69%)** |
| **Total** | **24** | **98** | **13** |

Interestingly neonates born with the help of Lower Segment - Caesarean Section (LSCS) had higher incidence of neonatal hyperbilirubinemia 53.85% than the Normal Vaginal Delivery (NVD) where the incidence was 46.15%.The causes for the Observed differences might be multifactorial viz., due to oxytocin induction and or spinal anaesthesia.The incidence of physiological jaundice and pathological jaundice was high i.e., 60.20% and 61.53% respectively in neonates with 2.5-3kg birth weight. While that trend started to decline to 35.7% and 30.77% respectively as the weight of neonates increased to 3-3.5 kg and then further declined to 4.08% and 7.69% respectively in 3.5-4 kg as shown in Table 1.

| Table 2: Association of NNH and Blood group of the neonate | | | |
|---|---|---|---|
| **Blood Group** | **No Jaundice** | **Clinical Jaundice** | **Pathological Jaundice** |
| **A+** | **9 (37.50%)** | **44 (44.89%)** | **6 (46.15%)** |
| **A-** | **1 (4.17%)** | **2 (2.04%)** | **0** |
| **B+** | **6 (25.00%)** | **52 (53.06%)** | **7 (53.85%)** |
| **B-** | **1 (4.17%)** | **0** | **0** |
| **AB+** | **5 (20.83%)** | **0** | **0** |
| **AB-** | **2 (8.33%)** | **0** | **0** |
| **Total** | **24** | **98** | **13** |

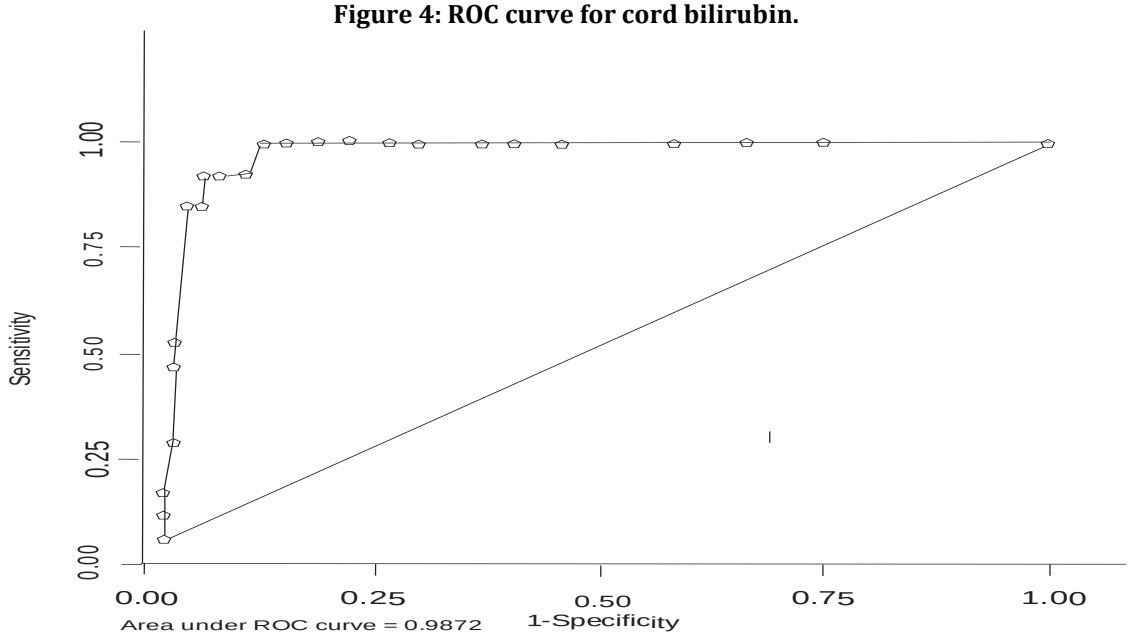
The association of Baby's blood group with Neonatal Hyperbilirubinemia is depicted in Table 2. Pathological Jaundice was found more in B+ blood group followed by A+ group. Physiological jaundice was also more in B+ followed by A+ blood group, though there is no marked difference. No strong association was found between blood group and incidence of NNH.

**Figure 3: Correlation of bilirubin level in umbilical cord and 4th day serum**




Umbilical cord venous blood bilirubin levels at birth for all 135 term new born and the follow up 4th day bilirubin levels were monitored and followed up for correlation of early prediction of neonatal Hyperbilirubinemia.
Bilirubin levels measured at two different time intervals in a span of few days demonstrated an excellent correlation coefficient r=0.86 (P-value < 0.001) as seen in Figure 3, which could be an assertive early predictive marker of elevated umbilical cord blood and serves as an indicator or determining factor screening the neonates for developing pathological jaundice (Neonatal Unconjugated Hyperbilirubinemia) in their early few days of life.

**Figure 4: ROC curve for cord bilirubin.**




| Table 3: Cut-off limits and Diagnostic utility of Total Umblical cord serum Bilirubin test | | | | | |
|---|---|---|---|---|---|
| **Purpose** | **Cut-off** | **Sensitvity (95% CI)** | **Specificity (95% CI)** | **Pvp** | **Pvn** |
| **Screening** | **≥2.30 mg/dL** | **92.3% (87.7, 96.7)** | **97.5% (92.7, 102.4)** | **92.3** | **97.5** |
| **Optimal** | **≥2.40 mg/dL** | **100.0% (80.0, 120.0)** | **88.6% (84.2, 93.0)** | **100.0** | **88.6** |
| **Diagnostic** | **≥3.00 mg/dL** | **100.0% (80.0, 120.0)** | **85.3% (81.1, 89.6)** | **100.0** | **85.3** |
| **Where Pvp-Positive Predictive Value; Pvn-Negative Predictive Value** | | | | | |

Predictive value of umbilical cord bilirubin levels as an early marker for development of NNH was obtained by receiver operating characteristics (ROC) analysis of cord bilirubin levels with regard to developing hyperbilirubinemia (pathological jaundice) at 4th day of new born life as depicted in Figure 4.

The specificity and sensitivity for a cut-off of ≥ 3mg/dL was calculated for umblical cord blood bilirubin and was found to be 93% and 97.6% respectively. Similarly the Positive Predictive Value (Pvp) was calculated to be 84.6% and Negative Predictive Value (Pvn) was 98.4%. The cord blood bilirubin levels predicted the development of hyperbilirubinemia at 4th day of birth, with an area under curve (AUC) of 0.982; P<0.001 as depicted in Table 3.

## Discussion:

Major cause for Pathological Hyperbilirubinemia is ABO incompatibility followed by Rh-incompatability. Reports of bilirubin induced brain damage in healthy term neonates even without hemolysis have increased the concern about Neonatal hyperbilirubinemia. A total of 135 neonates included in this study were term neonates with appropriate birth weight of 2.5-4kg. In this study cord blood bilirubin and 4th day serum bilirubin were evaluated and many risk factors ascertained to cause NNH, where 13 out of total 135 neonates developed pathological jaundice. The incidence is roughly 10% which is higher than some studies published elsewhere[13]. No strong association of NNH with sex (Male and Female) was found among the study population. These results were similar to the studies conducted abroad [14,15]. Incidence of Neonatal hyperbilirubinemia (physiological and pathological jaundice) was higher in neonates between 2.5-3kg than 3-4kg category. The study results were matching with studies done elsewhere [16, 17]. No strong association was found between mode of delivery and onset of Neonatal hyperbilirubinemia as in reports of other studies done in India and elsewhere [6, 14].

Correlation of Cord blood bilirubin values when compared to 4th day serum bilirubin assay indicated that screening, optimal and diagnostic cut-off to predict pathological jaundice progression was ≥2.3 mg/dL, ≥2.4 mg/dL and ≥3.0 mg/dL respectively. So a cord blood bilirubin level of 2.3 mg/dL should raise a suspicion that the neonate may show a paradigm shift to develop Pathological jaundice in the near future. And a CBBA value of ≥3.0mg/dL should be an alarming signal to observe the high risk neonate in the hospital for 4-5 days and phototherapy to be initiated as per AAP guidelines [18]. Serial bilirubin levels to be ascertained during the course of stay and a pre-discharge bilirubin assay to be compulsorily done. Other studies [15,19] reported that development of pathological NNH occurred when a cord blood bilirubin range was between 1.7-5mg/dL.

In present study, we also noticed that there was excellent correlation of CBBA levels to 4th day serum bilirubin in predicting pathological NNH. The results were similar to many studies done abroad [13, 20, 21]. All neonates with pathological jaundice received phototherapy and were discharged after treatment with no case fatalities.

**Limitations of the study:**

Frequent pricks for sampling babies to monitor Serum Bilirubin levels, Prolonged duration of stay, Lost to Follow-up because of early discharge of the baby and the mother against medical advice, Financial constraints for prolonged therapy for the child's family, Co-morbid disease conditions of the babies needing interventional therapy, getting prompt and accurate laboratory reports were the limitations of this study.

## Conclusion:

Cord blood bilirubin assay (CCBA) is an easy, feasible, non-invasive, cost-effective, time saving, early prediction marker to diagnose the development of Pathological Jaundice (Unconjugated Hyperbilirubinemia) in a setting of ABO at birth. These cases at risk of developing NNH could be observed for 3-4 days in the hospital and can be managed effectively with phototherapy as per AAP guidelines thereby preventing the devastating complications of NNH. A cord blood bilirubin at birth of ≥2.3mg/dl should raise a suspicion of evolving pathological hyperbilirubinemia in neonates. CBBA could be used as reliable early marker for NNH especially in developing and underdeveloped countries of the world.

## Recommendations:

1. Cord blood bilirubin could be used as an indicator of risk of icterus in newborns.
2. The use of cut-off cord bilirubin levels of 3mg/dl in healthy term neonates could be a useful predictor of significant Hyperbilirubinemia that will need phototherapy and avoid the risk of severe Hyperbilirubinemia that may need exchange transfusion.
3. Neonates with bilirubin level of ≥2.3mg/dL to be observed in NICU and monitored for Bilirubin levels 6th hourly and if needed prompt phototherapy to be given early.
4. Routine pre-discharge bilirubin and transcutaneous bilirubin could be used for prognosis and effective management during pre-phototherapy and post-phototherapy period.
5. Follow-up visits may be more helpful even if the neonate is discharged in 48 hours.

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