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A Study On Usefulness Of Cord Blood AnalysisIn Predicting Pathological Hyperbilirubinemia In Babies At Risk Of Developing ABO Incompatibility.

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ABSTRACT

Cord bilirubin and hemoglobin analysis helps not only in predicting the pathological jaundice in ABO incompatibility but also useful for early referral and intervention for better outcome. Aim of this study is to evaluate the cord blood bilirubin and hemoglobin analysis in predicting pathological hyperbilirubinemia in newborn at risk of ABO incompatibility. In this descriptive study conducted in Sri Muthukumaran Medical college, Chikkarayapuram, near Mangadu, Chennai, in the year 2022-2023. A positive or B positive babies born to O positive mothers with birth weight >2.5 kgs and gestational age >37 weeks were included. A total of 212 babies were studied. Cord bilirubin, reticulocyte count, hemoglobin and fourth day bilirubin were evaluated and data was analysed using Pearson's Chi square and ANOVA. 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. Babies with cord bilirubin >1.8mg/dl and hemoglobin <15.1gm/dl are more prone for pathological hyperbilirubinemia.
Keywords: ABO incompatibility, Jaundice, Cord bilirubin

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INTRODUCTION

Jaundice is the commonest abnormal finding in the first week of life. The clinical jaundice will manifest in neonates at a serum bilirubin level above 5.0 to 7.0 mg/dL (86 – 119 micromoles/L) 1. Chemical hyperbilirubinemia, which is defined as serum total bilirubin level of 2.0 mg/dL (34micromoles/L) or more, is virtually universal in newborns during first week of life [1]. Between 25 – 50 % of all term newborns and a higher percentage of premature infants develop clinical jaundice. Also 6.1 % of well term newborns have a maximal serum bilirubin level > 12.9mg/dL. A serum bilirubin level of > 15mg/dL is found in 3 % of normal term babies [2]. As the intensity of jaundice increases, there is cephalocaudal progression of yellow discoloration of skin. Hyperbilirubinemia can cause bilirubin encephalopathy and severe sequelae. So, it is imperative that pathological hyperbilirubinemia is picked up early and vigorous treatment is started [3]. When the newborn stays at the hospital for a 72-hour post-delivery period, it is possible to observe the peaking of the physiological jaundice, thus allowing medical intervention, if necessary [4]. However, in cases of early discharge from the hospital, the newborns may be subjected to re-admission for phototherapytreatment because of high levels of unconjugated bilirubin [5]. 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, brings emotional problems and risks to breast-feeding, and is one of the causes of early weaning [6].

Inclusion criteria

- A positive or B positive babies born to O positive mothers
- Gestational age of more than 37 weeks
- Birth weight of more than 2.5kgs
- Apgar of >7 at 1 minute of life

Exclusion criteria

Birth asphyxia, sepsis, birth trauma and babies born to preeclampsia and diabetic mothers. Written consent taken regarding the participation of the mother and baby in the study. Mothers with blood group O positive without hypertension/ diabetes and babies born to them with more than 7 Apgar were included in the study.

Demographic profile and relevant information of all the babies included in the study were collected using a structured proforma by interviewing the parents. Gestational age was assessed by the Modified new Ballard score. Babies were clinically assessed forage (hours/days), sex, gestational age, birth weight, previous jaundice in the family, day of onset of jaundice, pattern of feeding, fever and neurological symptoms like poor sucking, hypotonia and seizures. All babies were clinically assessed twice daily for presence and extent of jaundice based on Kramer criteria and for the appearance of any other illness. Visual inspection of jaundice was by examining a naked baby in bright natural light and in absence of yellow background. Thorough clinical examination of the baby was done with special emphasis on icterus, hepatosplenomegaly, extravasation of blood (cephalhematoma/sub-galeal bleed), excessive bruising, neurological signs of bilirubin induced neurological damage (BIND)Samples were taken for testing blood grouping and typing, serum bilirubin, hemoglobin and direct Coomb's test. Serum bilirubin was estimated by Diazo method. Reticulocyte count was estimated by supravital staining with brilliant cresol blue and hemoglobin estimated with autoanalyzer. Cord blood bilirubin was estimated, followed by serum bilirubin estimation in the neonate at 24, 48 and 72 hours of age. Cord blood (2 ml) was collected from placental side by free flow of blood from cord in an EDTA vial and clot vial within 1-2 min of birth and subjected to following investigations-(a) blood group by test tube method; and (b) total and direct serum bilirubin by calorimetric method. Venous blood (2 ml) sample was collected from peripheral vein of the baby at 24, 48 and 72 hours of life and subjected to following investigation. Serum bilirubin collected on fourth day of life of more than 15mg/dl was taken as pathological.

Statistical analysis

The data was analysed using SPSS version 17.0. Pearson Chi square test and ANOVA was used for comparison of multiple variables. Pearson correlation was used for finding correlation between variables and ROC curve used to define sensitivity and specificity of the variables.

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RESULTS

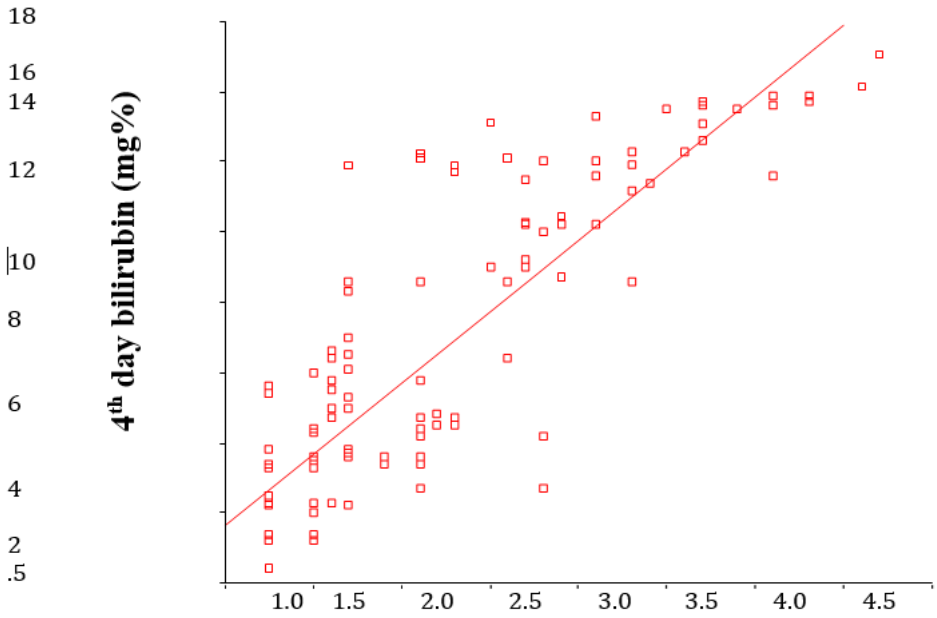
Out of the 136 babies who were at risk of ABO incompatibility, 73% (99) of the babies developed clinical jaundice and nearly 10% (13) of cases developed pathological jaundice. Out of the 136 babies studied 73 were males (54%) and 63 were females (46%). The incidence of clinical jaundice and pathologicalhyperbilirubinemia was not significantly different in both sexes (P=0.92). In our study group only babies between 2.5 to 4 kilograms were included (Appropriate for Gestational age)

Table 1: Blood Group As A Risk Factor

Blood group	No Jaundice	Jaundice	Pathological hyperbilirubinemia
A+	18	44	6
A-	2	2	0
B+	16	53	7
B-	1	0	0

Out of the 136 babies, 66 were A group (62 A positives and 4 A negatives) and 70 were B group (69 B positives and 1 B negative).Incidence of clinical jaundice B group (85%) was higher in compared to A group (74%). But this difference was not statistically significant (p value 0.91). No significant difference was there between the incidence of pathological hyperbilirubinemia also.

Graph :1 Correlation Between Cord Bilirubin And 4th DayBilirubin



Graph 1: Cord bilirubin has excellent correlation with the 4th day bilirubin levels. Pearson's correlation, r = 0.86 (p-value < 0.001).For who were started on phototherapy before the 4th day, a corresponding 4th day value based on nomagram was used for statistical analysis.

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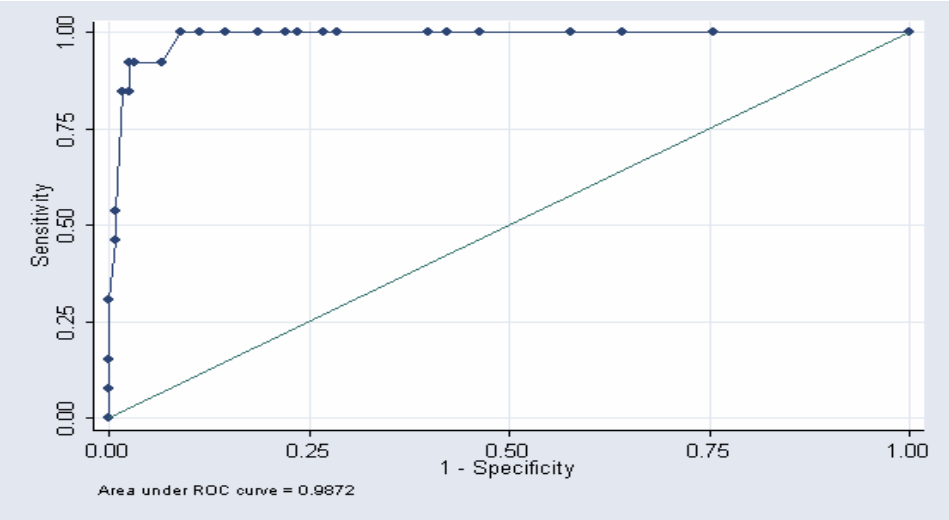
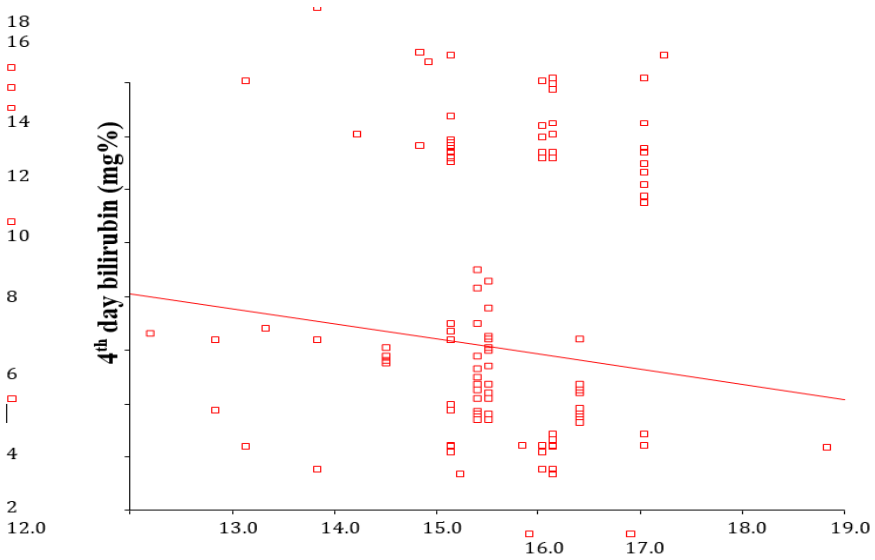
Graph 2: Receiver Operated Characteristic Curve



Graph 2: Area under the curve is 0.9872 which means that a randomly selected child with hyperbilirubinemia has a score larger than that for randomly chosen child without hyperbilirubinemia 98% of the time.A value of ≥ 3 mg/dL can be used as a cut off for predicting pathological hyperbilirubinemia with a specificity of 92.3%, sensitivity of97.5%, positive predictive value of 84.6 % and negative predictive value of 98.4 %

Graph 3: Cord Blood Hemoglobin And 4th Day Bilirubin



Hemoglobin g%

Lower cord hemoglobin is associated with higher risk of hyperbilirubinemia. The strength of the association is weak - Pearson's correlation r = - 0.139 (p-value=0.11).

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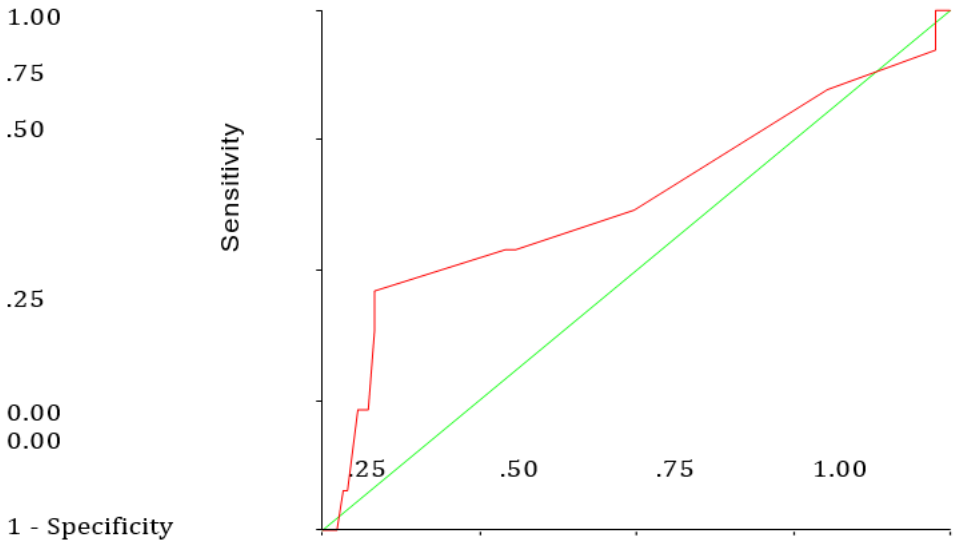
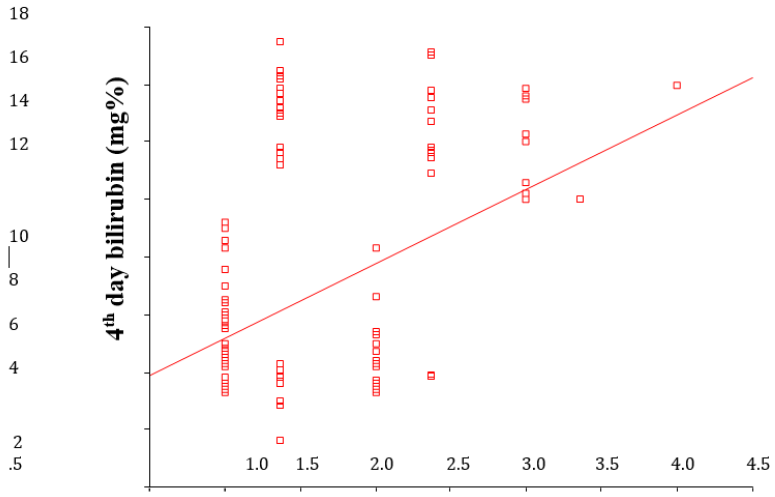
Graph 4: Receiver Operated Characteristic Curve For Hemogloblin



Graph 4: Area under the curve was .633. A hemoglobin value below 14.55g/dL can be used as a good predictor from this curve with a sensitivity of 53.8 %, specificity of 70.7 % positive predictive value of 15.9 % andnegative predictive value of 93.4 %

Graph 5: Correlation Between Reticulocyte Count And 4thDay Bilirubin



Graph 5: The risk of hyperbilirubinemia increases with an increase in reticulocyte count p-value = 0.002. The strength of the correlation was weak Spearman's correlation r = 0.364 (p-value <0.01).

Table 2: Direct Coombs Test And Pathological Hyperbilirubinemia

Direct coomb's test	Pathological hyperbilirubinemia	
	Present	Absent
Positive	2	0
Negative	11	123

Direct coomb's test was positive in only 1.5 % of the babies studied. It was positive in 15.4 % of babies who developed pathological hyperbilirubinemia. All children who had positive Direct coombs test developed pathological hyperbilirubinemia. P-value = 0.008

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DISCUSSION

The major cause of pathological hyperbilirubinemia is ABO incompatibility. This study was conducted to find out whether routine cord blood analysis can be useful in predicting pathological hyperbilirubinemia in newborns at risk of pathological hyperbilirubinemia. The aim was to find out whether cord blood bilirubin, hemoglobin and reticulocyte count values correlated with the peak bilirubin values. If these values could predict the development of pathological hyperbilirubinemia we could decide on the early discharge of these at risk babies. This study included 136 babies who were at risk of ABO incompatibility which include babies with either A or B blood group born to O positive mothers. All babies were term (>37 weeks) and appropriate for gestational age (2.5 – 4 kg). Those babies who had other potential causes for developing jaundice like birth asphyxia, sepsis, birth injuries, mother with diabetes, PIH were excluded from the study [7]. Preterm babies were excluded from the study because the serum bilirubin levels and the peak level going for kernicterus were highly variable. The aim was to find out cord blood analysis is useful in predicting pathological hyperbilirubinemia.[8] Factors in the cord blood that we studied included cord bilirubin, hemoglobin, reticulocyte count and direct coomb's test. Higher cord bilirubin levels, lower cord hemoglobin, higher reticulocyte count and a positive direct coomb's test were associated with a higher risk of the babies to develop pathological hyperbilirubinemia. In our study we tried to determine the correlation between cord blood bilirubin, hemoglobin, reticulocyte count and direct coomb's test positivity with the development of pathological hyperbilirubinemia.[9] Final outcome measurement is pathological bilirubinaemia which is defined in our study as a 4^{th} day bilirubin value above 15 mg/dL or a serum bilirubin level more than the 95^{th} percentile for the age in hours.13 out of 136 babies studied in our study developed pathological hyperbilirubinemia in our study (9.56 %) [10]. Bilirubin peaking was mostly noted in the 3^{rd} and 4^{th} day. Out of the 13 babies who had pathological hyperbilirubinemia 12 required phototherapy (phototherapy started on 3^{rd} or 4^{th} day) and 1 required exchange transfusion (on 3^{rd} day of life). The baby who required exchange transfusion had a cord blood bilirubin of 4.2mg/dL, cord blood hemoglobin 13.2mg/dL. But her reticulocyte count was 1 % and direct coomb's test was negative.[11] Cord bilirubin values in the study population were in the range of <1 to 4.2mg/dL, while that in the babies who developed hyperbilirubinemia was in the range of 2 – 4.2 mg/dL. The mean bilirubin level in babies with pathological hyperbilirubinemia was 3.1 mg/dL, where as those who didn't develop it was 1.31 mg/dL (p value <0.01) [12]. Cord hemoglobin values in the study population were in the range of 12.2 to 18.2 mg/dL, while that in the babies who developed hyperbilirubinemia was in the range of 12.5 – 16.6 mg/dL. The mean hemoglobin level in babies with pathological hyperbilirubinemia was 14.73 mg/dL, where as those who didn't develop it was 14.62 mg/dL (p value 0.92). Hemoglobin was a poor predictor for the development of pathological hyperbilirubinemia [13]. Reticulocytosis in ABO hemolytic disease range from 6 % to 40 % in various studies Reticulocytosis is seen in cord blood itself as the hemolysis in ABO incompatibility starts in utero itself. But in our study significant reticulocytosis was seen in none of the babies. Direct coomb's test is usually negative or weakly positive in babies with babies with ABO incompatibility [14]. In our study only two babies with pathological hyperbilirubinemia had Direct coombs test positivity (15.4%). None of the babies without pathological hyperbilirubinemia had Direct coombs test positive. Previous studies conducted showed a good correlation between cord blood bilirubin and the development of pathological hyperbilirubinemia. The cord bilirubin values which can predict pathological hyperbilirubinemia range from 1.7 mg/Dl [15]. In our study cord bilirubin has excellent correlation with the 4^{th} day bilirubin levels Pearson's correlation, r = 0.86 (p-value < 0.001). So cord bilirubin can effectively predict the risk of pathological hyperbilirubinemia. Lower cord hemoglobin is associated with higher risk of hyperbilirubinemia. The strength of the association is weak – Pearson's correlation r= - 0.139 (p-value=0.11) [16]. The risk of hyperbilirubinemia increases with an increase in reticulocyte count p-value = 0.002. The strength of the correlation was weak Spearman's correlation r= 0.364 (p- value <0.01). But in our study the reticulocytosis in babies with ABO incompatibility was not in the pathological range for the newborns. DIRECT COOMBS TEST was positive in only 1.5 % of the babies studied [17]. It was positive in 15.4 % of babies who developed pathological hyperbilirubinemia. All children with positive DIRECT COOMBS TEST developed pathological hyperbilirubinemia. P-value = 0.008.A cord bilirubin value of $\geq$ 3mg/dL can be used as a cut off for predicting pathological hyperbilirubinemia with a specificity of 92.3%, sensitivity of 97.5%, positive predictive value of 84.6 % and negative predictive value of 98.4% [18]. A hemoglobin value below 14.55 g/dL can be used as a good predictor from this curve with a sensitivity of 53.8 %, specificity of 70.7 %, positive predictive value of 15.9 % and negative predictive value of 93.4 %. A reticulocyte count $\geq$2% could predict the risk of pathological hyperbilirubinemia with a sensitivity of 46 % and specificity of 67%. Direct coomb's test is positive in 15.4 % of babies who developed pathological hyperbilirubinemia. All children who had positive DIRECT COOMBS TEST developed

pathological hyperbilirubinemia. (P-value=0.008) [19,20].

CONCLUSION

Cord blood analysis is useful for predicting pathological hyperbilirubinemia in babies at risk of ABO incompatibility. In cord blood analysis cord bilirubin is the best predictor for the development of hyperbilirubinemia.Neonates with cord bilirubin values ≥ 3 mg/dL are at higher risk for developing pathological hyperbilirubinemia. A lower cord blood hemoglobin level is associated with a higher 4th day bilirubin level. A cord hemoglobin level below 14.55 g/dL was associated with a higher risk of babies to develop pathological hyperbilirubinemia. Significant reticulocytosis in cord blood was not seen in babies with the risk of ABO incompatibility. Though Direct coomb's test was positive only 15 % of babies with pathological hyperbilirubinemia, all babies with positive direct coomb's test developed pathological hyperbilirubinemia.

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