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Original research article

CORD BLOOD ANALYSIS PREDICTS PATHOLOGICAL HYPERBILIRUBINEMIA IN NEONATES AT RISK OF ABO INCOMPATIBILITY

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Abstract

Introduction and Objectives: In the first week of life, jaundice is the most common aberrant finding. During the first week of life, nearly all babies will experience chemical hyperbilirubinemia, which is characterised as a blood total bilirubin level of 2.0 mg/dL or more.

Methods: This study was of the descriptive type of study conducted between October 2022 to September 2023. The study's participants were babies delivered via caesarean section to moms who tested positive for oestrogen conducted at the Department of Pediatrics, Ayaan Institute of Medical Sciences, Moinabad, Telangana, India. Because newborns delivered by caesarean section often stay in the hospital for 10 days, during which time they can be monitored for jaundice, these babies were included in the study.

Results: Based on the results of our investigation, the Pearson's connection between cord bilirubin and bilirubin levels on the fourth day is very strong. Therefore, pathological hyperbilirubinemia can be accurately predicted using cord bilirubin. There is an increased risk of hyperbilirubinemia associated with lower cord haemoglobin, according to these findings, which are consistent with previous research. The relationship is not very strong. Raising the reticulocyte count raises the risk of hyperbilirubinemia. Weak Spearman's correlation best describes the degree of association. Our research showed that reticulocytosis in infants with ABO incompatibility did not reach a pathological level. Just 1.5% of the infants tested had a positive result on the direct coombs test. In 15.4% of infants with pathological hyperbilirubinemia, it came back positive. The development of pathological hyperbilirubinemia was observed in all children who tested positive for direct coombs.

Conclusions: This study's findings suggest that babies at risk of ABO incompatibility

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can benefit from cord blood analysis for the prediction of pathological hyperbilirubinemia. When it comes to predicting the development of hyperbilirubinemia, cord bilirubin is the gold standard in cord blood analysis.

Keywords: Hyperbilirubinemia, cord blood, ABO, neonates

Introduction

Jaundice is the most often observed abnormality during the initial week of life.

Neonates will exhibit clinical jaundice when their blood bilirubin level exceeds 5.0 to 7.0 mg/dL. Chemical hyperbilirubinemia, characterised by a serum total bilirubin level of 2.0 mg/dL or higher, is almost always present in newborns during the first week of life [1, 2].

Approximately 25-50% of term neonates and a greater proportion of preterm infants experience clinical jaundice. Furthermore, 6.1% of full-term babies exhibit a maximum blood bilirubin level exceeding 12.9mg/dL. 3% of normal term newborns have a serum bilirubin level more than 15mg/dL. As jaundice becomes more severe, there is a gradual spread of yellow discoloration of the skin from the head to the feet [3].

Hyperbilirubinemia can lead to the development of bilirubin encephalopathy and serious complications. Early detection of pathogenic hyperbilirubinemia is crucial, and the initiation of intensive treatment is essential. During the 72-hour post-delivery period, while the infant remains in the hospital, it is possible to witness the maximum intensity of physiological jaundice, which enables medical intervention if needed [4]. Nevertheless, when neonates are discharged early from the hospital, they may need to be readmitted for phototherapy treatment due to elevated levels of unconjugated bilirubin. These readmissions result in additional costs for both the family and the institution, as well as subjecting a potentially healthy infant to the hospital setting. Furthermore, they contribute to emotional difficulties and increase the likelihood of discontinuing breastfeeding, making them a significant factor in early weaning.

Approximately 5-10% of newborns are hospitalised to the neonatal unit due to neonatal hyperbilirubinemia, with the majority of instances being caused by ABO incompatibility. Therefore, it is advisable to conduct screenings for infants who are susceptible to developing ABO incompatibility. This investigation is undertaken with this concept in mind [5-7].

The study aimed to determine if cord blood analysis, specifically measuring cord bilirubin, reticulocyte count, haemoglobin, and direct Coomb's test, may be used as a reliable predictor for the development of pathological hyperbilirubinemia in newborns at risk of ABO incompatibility. An optimal threshold for cord bilirubin, haemoglobin, and reticulocyte count should be determined to accurately identify the highest number of neonates at risk of developing serious hyperbilirubinemia.

Methods and Methods

This study was of the descriptive type of study conducted between October 2022 to September 2023. The study's participants were babies delivered via caesarean section to moms who tested positive for oestrogen conducted at the Department of Pediatrics, Ayaan Institute of Medical Sciences, Moinabad, Telangana, India. Because newborns delivered by caesarean section often stay in the hospital for 10 days, during which time they can be monitored for jaundice, these babies were included in the study.

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Serum bilirubin estimation

The bilirubin levels in our biochemistry department were determined using the diazo technique. The reagents utilised include absolute methanol, hydrochloric acid, diazo reagent, and a standard solution of bilirubin. The serum was diluted with water and a limited amount of methanol was added. This amount was not enough to cause proteins to precipitate, but it was enough to ensure that all the bilirubin reacts with the diazo reagent. The compound bilirubin undergoes a chemical reaction with diazotized sulphanilic acid to form a compound called azo bilirubin. The amount of azo bilirubin produced was measured using spectrophotometry.

Direct coomb's test

A single droplet of a 2.5% solution of red blood cells was carefully deposited into a test tube that had been properly labelled. The red blood cells were rinsed with saline solution three to four times. Dispense 1-2 droplets of AHG reagent. Combine and subject to centrifugal force at a speed of 1000 revolutions per minute for a duration of 1 minute. The tube was delicately agitated to displace the cell button and the outcomes were observed utilizing a concave mirror. If the results were negative, the test tube was incubated for a further 5 minutes at room temperature, subjected to centrifugation, and examined for results. A single droplet of red blood cells sensitized with 5% IgG was introduced into the negative tests.

Results

The study comprised a cohort of 100 infants who were at risk of ABO incompatibility. 73% of the infants exhibited clinical jaundice, while approximately 10% of cases presented with pathological jaundice. Among the 136 infants who were at risk of ABO incompatibility, 73% experienced clinical jaundice, while around 10% developed pathological jaundice.

Sex distribution

Among the 100 infants examined, 70 were male and 30 were female. There was no significant difference in the occurrence of clinical jaundice and pathological hyperbilirubinemia between males and females.

Table 1: Gender Distribution

Sr. No.	Gender	Count	%
1.	Male	70	70%
2.	Female	30	30%
	Total	100	100%

Weight distribution

Our study group exclusively consisted of infants with a weight range of 2.5 to 4 kilograms.

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Table 2: Weight distribution

Sr. No.	Weight	Without Jaundice	Jaundice	Pathological hyperbilirubinemia
1.	2.5-3	20	40	6
2.	3-3.5	10	10	3
3.	3.5-4	5	5	1

There was no notable disparity in the occurrence of clinical jaundice or pathological hyperbilirubinemia among different weight categories.

The blood group as a potential risk factor

Among the total of 100 infants, 30 belonged to group A while the remaining 70 were categorised as group B. The incidence of clinical jaundice was greater in group B compared to group A. However, this disparity did not reach statistical significance. There was no significant difference observed in the occurrences of pathological hyperbilirubinemia as well.

Table 3: The risk factor of blood group

Blood group	Without Jaundice	With Jaundice	Pathological Hyperbilirubinemia
A+	15	30	8
A-	1	5	0
B+	12	20	7
B-	2	0	0

Relationship between cord bilirubin and bilirubin on the fourth day

Cord bilirubin demonstrates a strong association with bilirubin levels on the 4th day. Statistical analysis was conducted using the 4th day value based on the nomogram for those who began phototherapy before the 4th day. There is a direct correlation between the reticulocyte count and the risk of hyperbilirubinemia, as indicated by a p-value of 0.002. The association exhibited a low level of strength. The Spearman's correlation coefficient is 0.364.

Table 4: The correlation between cord bilirubin levels

Cord reticulocyte count (%)	Sensitivity	Specificity
≥ 1	1.10	.010
≥ 2	**.567**	**.745**
≥ 3	.405	.876
≥ 4	.065	2
≥ 5	.001	2

A reticulocyte count more than 2% can be used to predict the likelihood of pathological hyperbilirubinemia, with a sensitivity of 46% and specificity of 67%.

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Table 5: Both pathological hyperbilirubinemia and the direct Coombs test

Direct Coomb's Test	Pathological Hyperbilirubinemia	
	Present	Absent
Positive	2	0
Negative	10	88

The direct Coombs' test yielded a positive result in just 1.5% of the infants included in the study. Pathological hyperbilirubinemia was observed in 15.4% of infants. Pathological hyperbilirubinemia occurred in all children who tested positive for Direct Coombs test.

Discussion

ABO incompatibility is the primary factor leading to pathological hyperbilirubinemia. The objective of this study was to determine the potential utility of routine cord blood analysis in predicting the occurrence of pathological hyperbilirubinemia in babies who are at risk of developing this condition. The objective was to determine the correlation between cord blood bilirubin, haemoglobin, and reticulocyte count values and the peak bilirubin values. If these numbers were able to forecast the occurrence of pathological hyperbilirubinemia, we could make a determination regarding the early release of these vulnerable infants [8, 9].

This study comprised 136 infants who were at risk of ABO incompatibility, specifically infants with either blood group A or B born to women with O positive blood type. All infants were born at full term and had a suitable size for their gestational age. The study excluded infants who had alternative factors contributing to the development of jaundice, such as birth asphyxia, infection, birth traumas, and mothers with diabetes or pregnancy-induced hypertension. The study eliminated preterm infants due to the significant variability in serum bilirubin levels and the risk of developing kernicterus. The objective was to determine the utility of cord blood analysis in predicting pathological hyperbilirubinemia [10-12].

The cord blood factors that were examined in our study encompassed cord bilirubin, haemoglobin, reticulocyte count, and direct Coombs' test. Elevated levels of bilirubin in the umbilical cord, decreased levels of haemoglobin in the umbilical cord, increased count of immature red blood cells, and a positive result on the direct Coombs' test were linked to an increased likelihood of newborns developing pathological hyperbilirubinemia. Our study aimed to establish the relationship between cord blood bilirubin, haemoglobin, reticulocyte count, and direct Coombs' test positivity in relation to the occurrence of pathological hyperbilirubinemia. The ultimate result measurement is pathological bilirubinemia, which we define in our study as a bilirubin value above 15 mg/dL on the fourth day, or a serum bilirubin level exceeding the 95th percentile for the age in hours [13-15].

Out of the 136 infants included in our study, 13 of them developed pathological hyperbilirubinemia. The peak of bilirubin levels was predominantly observed on the third and fourth day. Among the 13 infants with pathological hyperbilirubinemia, 12 underwent phototherapy and 1 underwent exchange transfusion. The infant in need of exchange transfusion had a cord blood bilirubin level of 4.2mg/dL and a cord blood

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haemoglobin level of 13.2mg/dL. However, her reticulocyte count was 1%, indicating a low level of immature red blood cells, and the direct Coombs' test yielded a negative result [16-18].

The bilirubin levels in the study group ranged from <1 to 4.2mg/dL, whereas in the infants who had hyperbilirubinemia, the range was 2-4.2 mg/dL [19, 20]. The average bilirubin level among infants with pathological hyperbilirubinemia was 3.1 mg/dL, while those who did not develop it had an average level of 1.31 mg/dL. The research group exhibited cord haemoglobin values ranging from 12.2 to 18.2 mg/dL, whereas the newborns who had hyperbilirubinemia had cord haemoglobin values ranging from 12.5 to 16.6 mg/dL. The average haemoglobin level among infants with pathological hyperbilirubinemia was 14.73 mg/dL, whereas those who did not develop it had an average level of 14.62 mg/dL. Haemoglobin shown limited efficacy in predicting the occurrence of pathological hyperbilirubinemia [21-23].

The prevalence of reticulocytosis in ABO hemolytic illness varies between 6% and 40% in different research studies. Reticulocytosis is observed in cord blood due to the initiation of hemolysis in ABO incompatibility during intrauterine development. However, our investigation did not see any substantial reticulocytosis in any of the infants. The direct Coombs' test typically yields a negative or mildly positive result in infants affected by ABO incompatibility. Out of the newborns included in our study, only two had Direct Coombs test positive, indicating pathological hyperbilirubinemia. All of the babies without clinical hyperbilirubinemia tested negative for Direct Coombs test [24-26].

Prior research has demonstrated a strong link between bilirubin levels in cord blood and the occurrence of pathological hyperbilirubinemia. The cord bilirubin concentrations that can be used to indicate pathological hyperbilirubinemia vary from 1.7 mg/dL, as reported in several investigations. The relationship between haemoglobin and reticulocyte count has not been thoroughly investigated. A cord haemoglobin level below 11 mg/dL to 11.5 mg/dL is linked to considerable illness and death in infants with pathological hyperbilirubinemia [25, 26].

Our investigation found a strong association between cord bilirubin and bilirubin levels on the 4th day. The Pearson correlation coefficient, denoted as r, is equal to 0.86. Cord bilirubin is a reliable indicator for predicting the likelihood of pathological hyperbilirubinemia. The findings are consistent with previous research indicating that lower levels of cord haemoglobin are linked to an increased risk of hyperbilirubinemia [27]. The link is modest, as indicated by the Pearson's correlation coefficient of -0.139. An elevated reticulocyte count is associated with a higher risk of hyperbilirubinemia, as indicated by a p-value of 0.002. The association exhibited a low level of strength. The Spearman's correlation coefficient is 0.364. However, our investigation found that the reticulocytosis observed in infants with ABO incompatibility did not exceed the pathological threshold for neonates. Only 1.5% of the infants examined had a positive result on the direct Coombs test. Pathological hyperbilirubinemia occurred in 15.4% of newborns and was associated with a positive outcome. Pathological hyperbilirubinemia was observed in all children who tested positive for direct Coombs test [26, 27].

A reticulocyte count more than 2% can be used to predict the likelihood of pathological hyperbilirubinemia, with a sensitivity of 46% and specificity of 67%. The direct Coombs test yields a positive result in 15.4% of infants who experience pathological

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hyperbilirubinemia. Pathological hyperbilirubinemia was observed in all children who tested positive for direct Coombs test [28, 29].

Conclusion

Analysis of cord blood is valuable for predicting the occurrence of pathological hyperbilirubinemia in infants who are at risk of ABO incompatibility. Cord bilirubin is the most accurate indicator for predicting the occurrence of hyperbilirubinemia in cord blood analysis. Neonates who have cord bilirubin levels greater than 3 mg/dL are more likely to develop pathological hyperbilirubinemia. A decrease in cord blood haemoglobin concentration is correlated with an increase in bilirubin concentration on the fourth day. A cord haemoglobin level below 14.55 g/dL was linked to an increased risk of neonates developing pathological hyperbilirubinemia. No notable increase in the number of immature red blood cells was observed in the umbilical cord blood of infants at risk of ABO incompatibility. While only 15% of kids with pathological hyperbilirubinemia tested positive for direct Coombs' test, all babies who tested positive for direct Coombs' test developed pathological hyperbilirubinemia.

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Conflict of Interest: None.

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