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**Original Research Article**

# Cord blood nucleated RBCs as a potential tool in prediction of neonatal hyperbilirubinemia in ABO incompatibility susceptible neonates

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## ABSTRACT

**Background:** Neonatal hyperbilirubinemia is one of the most commonly encountered conditions in neonatal period. Early recognition and prompt intervention can prevent its dreadful complication like bilirubin encephalopathy. nucleated red blood cells (nRBC) are released into the peripheral circulation in conditions causing oxidative stress. This study aims at finding the relation between nRBC and neonatal hyperbilirubinemia.
**Methods:** This hospital based prospective cohort study was conducted on 100 neonates. The neonates were divided into 2 groups: Group 1- cases (n=50) included neonates born to O positive mothers with A/B blood groups and Group 2-controls (n=50) with neonates born to O positive mothers with O blood group. The cord blood nRBC, TSB at 48 hours and requirement of phototherapy were the variables which were considered. Statistical analysis was done using SPSS 22 version software with appropriate statistical methods applied.
**Results:** The mean cord nRBC was 7.26±2.65 (Group 1) and 3.04±0.92 (Group 2) respectively. ROC curve for cord nRBC revealed AUC 0.944 at 4.0 cut-off with sensitivity of 92%, specificity of 94%, LR+15.33 and LR-6.085 with p<0.0001.
**Conclusions:** Cord blood nucleated RBCs is a simple, easily available test to predict the neonatal hyperbilirubinemia in ABO incompatibility susceptible neonates.

**Keywords:** ABO incompatibility, nRBC, Neonatal hyperbilirubinemia, Phototherapy

## INTRODUCTION

Neonatal hyperbilirubinemia is mostly benign and resolve unprompted. But it can lead to dreadful complications like kernicterus which can ultimately result in significant morbidity.[1] There are several causes for indirect hyperbilirubinemia such as escalated enterohepatic circulation, reduced conjugation of bilirubin, defective hepatic uptake and increased production of bilirubin due to hemolysis.[2,3] Although Rh incompatibility comprise the majority of hemolytic disease of newborn, incompatibility of ABO blood group is not rare. ABO incompatibility is seen when a mother with O blood group carries a fetus with a different blood type (type A, B, or AB).[4]

Due to brief period of postpartum stay in hospital, the identification and timely management of this condition is of prime importance.[5] Even the healthy term neonates are readmitted after discharge due to neonatal hyperbilirubinemia.[6,7] Nucleated RBCs are the predecessors of RBC which are seen in peripheral circulation in newborn period.[8,9] The number of nRBCs in peripheral smear can vary in different conditions.[8] Some conditions like birth asphyxia, blood loss/hemolysis, maternal diabetes, intrauterine infections, intrauterine growth restriction and preeclampsia can cause surge in the nucleated RBCs into circulation.[10] The pro-oxidant nature of bilirubin is dreadful than its antioxidant property.[11] This study is taken up to study the importance of cord blood nucleated RBCs in neonatal hyperbilirubinemia.

## METHODS

This prospective cohort study was undertaken in the postnatal unit of our hospital. Healthy term neonates,>37 weeks of gestation born via normal or cesarean section to mothers with O positive blood group were considered for the study. This study was conducted during the period September 2020 to January 2021. Informed consent was taken from parents. Neonates with positive direct Coombs test, presence of major congenital anomalies, Rh incompatibility, glucose-6-phosphate dehydrogenase deficiency, inborn error of metabolism, sepsis, multiple gestation, maternal history of diabetes, perinatal asphyxia, placenta previa and abruption were excluded. Gestational age, birth weight, gender, type of delivery, blood group types of infants and history of neonatal hyperbilirubinemia in siblings were taken into account. Under aseptic precautions 2cc cord blood sample in an EDTA (Ethylenediaaminetetraacetic acid) vacutainer was withdrawn from the neonate at delivery and peripheral smear examination was made immediately. Total serum bilirubin was measured on day 3 of life under aseptic precautions and analyzed immediately in Merilyzer auto quant 200 excelus which works on principle of diazo with sulphanilic acid method. The study group was categorized into two groups: group 1 consisting of neonates born to O positive mothers with A/B type blood group and group 2 including neonates born to O positive mothers with O type blood group. Outcome variable was need for phototherapy.

Categorical data was represented in the form of frequencies and proportions. Continuous data was represented as mean and standard deviation. Student t test was used to find the significance of study parameters on continuous scale between two groups (inter group analysis) on metric parameters. Leven`s test for homogeneity of variance was performed to assess the homogeneity of variance. p value of<0.05 was considered as statistically significant after assuming all the rules of statistical tests. Chi-square/Fisher exact test was used to find the significance of study parameters on categorical scale between two groups. Statistical analysis was done with SPSS 22.0

## RESULTS

Among 100 eligible newborn infants considered in the study, 52% infants were male and 51 infants were born via normal vaginal delivery. 50 infants had history of jaundice in previous sibling. There were no significant differences in baseline characteristics like gestational age, gender, delivery type and history of neonatal hyperbilirubinemia in siblings between group 1 (cases) and group 2 (controls) as presented in Table 1.

The collation of cord blood complete blood count parameters, nRBC, total serum bilirubin level at 48 hours of life and need of phototherapy at 48 hours of life are depicted in Table 2. Cord blood complete blood counts were not statistically different between the groups.

**Table 1: Clinical parameters.**

| | Group 1 (n=50) | Group 2 (n=50) | P value |
|---|---|---|---|
| **Gestational age** | 38.34±1.04 weeks | 38.30±1.03 | 0.848 |
| **Gender (male/female)** | 26/24 | 26/24 | 1 |
| **Delivery type (normal/Cesarean)** | 26/24 | 25/25 | 0.841 |
| **Jaundice in previous sibling** | 26 | 24 | 0.689 |

**Table 2: Laboratory parameters.**

| | Group 1 (n=50) | Group 2 (n=50) | P value |
|---|---|---|---|
| **Hemoglobin (g/dl)** | 17.47±2.11 | 17.77±1.95 | 0.469 |
| **WBC (/mm³)** | 17922.32±2539.89 | 17836.02±2541.57 | 0.865 |
| **Platelet (/mm³)** | 3.19±0.98 | 3.26±1.00 | 0.756 |
| **Cord nRBC (/100WBC)** | 7.26±2.65 | 3.04±0.92 | <0.001 |
| **TSB at 48 hours** | 15.46±3.03 | 12.20±2.19 | <0.001 |
| **Phototherapy** | 33 | 6 | ≤0.001 |

**Table 3: ROC curve analysis.**

| Variables | ROC results to predict Mortality | | | | Cut-off | AUROC | SE | P value |
|---|---|---|---|---|---|---|---|---|
| | Sensitivity | Specificity | LR+ | LR- | | | | |
| **nRBC/1000** | 92.0 | 94.0 | 15.33 | 0.085 | >4.0 | 0.944 | 0.026 | <0.001** |
| **TSB @ 48 hrs** | 72.0 | 88.00 | 6.0 | 0.32 | >14.1 | 0.809 | 0.044 | <0.001** |

Statistically significant differences were obtained in cord blood nRBCs, total serum bilirubin (TSB) and need for phototherapy between the groups ($p<0.001$, $<0.001$ and $\leq 0.001$ respectively). The nRBC count, total serum bilirubin levels and need for phototherapy were higher in the group 1 (cases) infants. The AUC from ROC were 0.944 for nRBC count and 0.809 for TSB at 48 hours (Figure 1, 2). The sensitivity and specificity of cord nRBC was higher than that of TSB at 48 hours (Table 3).

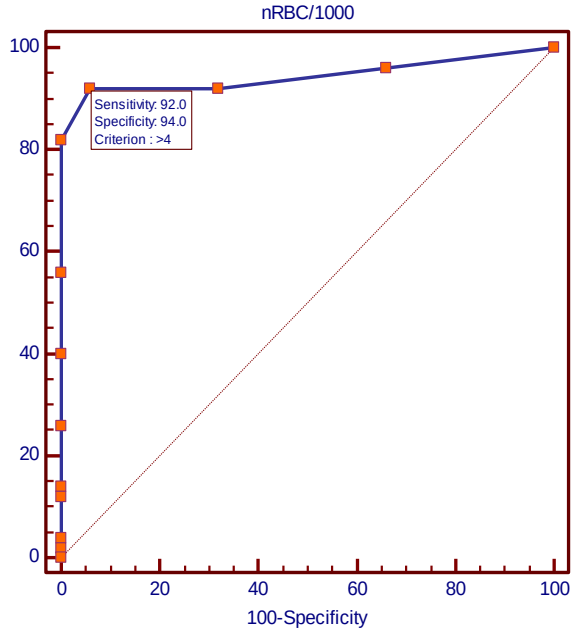


**Figure 1: Evaluation of accuracy of NRBC.**

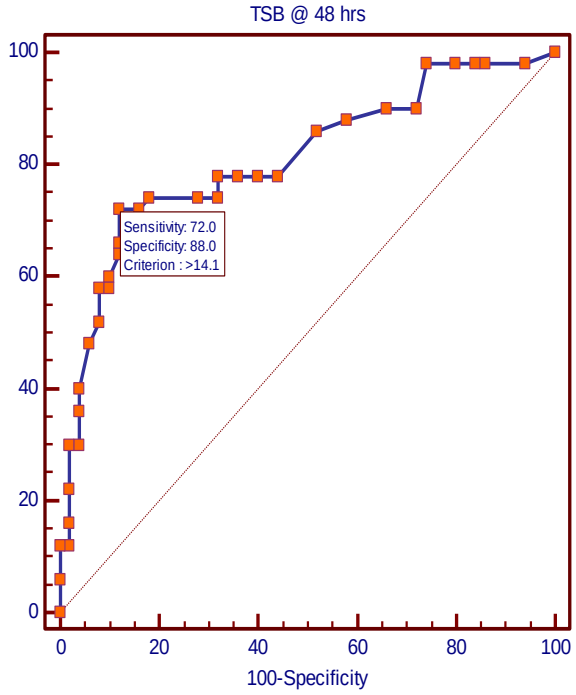


**Figure 2: Evaluation of accuracy of TSB at 48 hours**

## DISCUSSION

Unlike HDN due to Rh incompatibility, ABO incompatibility is less severe due to the reasons like decreased expression of ABO blood group antigens on fetal RBCs as compared to those in adults and due to its widespread distribution in different organs unlike adults wherein they are concentrated in RBCs.[4] Nevertheless, permanent brain damage due to bilirubin encephalopathy has been documented due to severe hyperbilirubinemia. It is of prime importance to identify and treat the condition early as to prevent this dreadful complication. Hence to avoid bilirubin encephalopathy, it is recommended to routinely ascertain the clinical risk factors for severe hyperbilirubinemia and measure bilirubin levels before discharge.[13] In our study, nRBCs in cord blood were significantly higher in newborns that required phototherapy and hence cord blood nRBCs can be a marker in prediction of development of neonatal hyperbilirubinemia. Many studies have shown that oxidative stress trigger hyperbilirubinemia in neonates.[14,15] In response to this there is induction of heme-oxygenase enzyme which further alleviates the production of bilirubin.[16–18] A negative Direct Antiglobulin test does not rule out ABO incompatibility.[19] The increased nucleated RBCs in cord blood can predict neonatal hyperbilirubinemia and need of phototherapy in neonates susceptible for ABO incompatibility.

## CONCLUSION

The estimation of cord blood nucleated RBCs is a simple, non-invasive and cost effective method which can aid in early identification of neonatal hyperbilirubinemia.

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