

Alper Orhon, Hatice Topal, Nilay Hakan and Esra Arun Ozer\*

# Cord blood nucleated red blood cell level: is it a predictive marker for neonatal jaundice?

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## Abstract

**Background:** The aim of this study was to evaluate if the cord blood nucleated red blood cell (nRBC) levels can predict the development of hyperbilirubinemia in healthy neonates.

**Methods:** All healthy newborn infants born after 35 or more weeks of gestation at our hospital between January 2016 and April 2017 were included. The levels of nRBC were counted in umbilical cord blood samples. Neonates were grouped into two study groups based on the presence or absence of neonatal jaundice.

**Results:** The study included overall 149 eligible newborn infants. The levels of nRBC and absolute nRBC count showed statistically significant differences between newborns with or without jaundice ( $p=0.01$  and  $0.02$ , respectively).

**Conclusions:** We suggest that increased nRBC counts in cord blood may be a predictive marker for hyperbilirubinemia in healthy newborn infants.

**Keywords:** bilirubin; cord blood; newborn; nucleated red blood cell.

## Introduction

Although indirect hyperbilirubinemia in the neonatal period is usually a benign condition that resolves spontaneously, the follow-up of jaundiced babies due to permanent brain damage caused by high bilirubin levels is of clinically great importance [1]. Neonatal hyperbilirubinemia results from a number of mechanisms

including increased enterohepatic circulation, impaired conjugation of bilirubin, deficiency of hepatic uptake and increased production of bilirubin [2, 3]. Short postpartum hospital stays have become a common practice [4]. It is evident that hyperbilirubinemia is the most common cause for readmission in the early neonatal period [5, 6]. Severe hyperbilirubinemia and bilirubin encephalopathy can occur in healthy, term neonates who were discharged early [7]. Thus, it is very important to predict which infants carry high risk for significant hyperbilirubinemia.

Nucleated red blood cells (nRBCs) are precursors of erythrocytes and frequently found in the peripheral blood of neonates and the number of nRBCs varies widely at birth [8, 9]. It has been shown that the number of nRBCs is increased in perinatal problems such as fetal hypoxia [10]. Increased nRBC counts may occur in different conditions other than fetal hypoxia such as fetal erythropoiesis, blood loss and hemolysis, maternal diabetes, intrauterine infections, intrauterine growth restriction and preeclampsia [8].

Bilirubin is an important antioxidant at physiological limits, but acts as a stronger pro-oxidant at higher levels. It is generated by the degradation of heme, whose source is red blood cells. The investigation of relation between hyperbilirubinemia and the increase of nRBCs in perinatal problems may be therefore of great importance. The aim of this study is to evaluate whether the cord blood nRBC can predict the development of hyperbilirubinemia in healthy neonates.

## Materials and methods

### Study participants

This prospective trial was performed at the well-infant nursery of our university hospital. All healthy newborn infants born after 35 or more weeks of gestation between January 2016 and April 2017 were enrolled into the study. The exclusion criteria were refusal to give informed consent, a positive direct antiglobulin test result, presence of major congenital or hematopoietic anomalies, Rh and/or ABO incompatibility, glucose-6-phosphate

\*Correspondence: Esra Arun Ozer, MD, Professor of Pediatrics, Celal Bayar University, School of Medicine, Department of Pediatrics, Manisa 45040, Turkey, E-Mail: [esra.arun@gmail.com](mailto:esra.arun@gmail.com)

Alper Orhon, Hatice Topal and Nilay Hakan: Mugla Sitki Kocman University, School of Medicine, Department of Pediatrics, Mugla, Turkey

dehydrogenase deficiency, inborn error of metabolism, sepsis, multiple gestation, maternal history of diabetes or smoking, and drug abuse, perinatal asphyxia, fetal distress or anemia, and placenta previa, abruption or infarcts. This study was approved by the local Ethical Committee of our institution and informed consent was obtained from the parents before enrollment.

## Demographic and clinical data

Gestational age, birth weight, gender, type of delivery, blood group types of parents and infants and history of neonatal hyperbilirubinemia in siblings were recorded. Body weight was measured on postnatal days 7 and 15. The total bilirubin levels of the babies were measured with a transcutaneous device (Drager Jaundice Meter JM-103, Drager Medical, Inc, Telford, PA, USA) on postnatal days 1, 3, 7 and 15.

Three groups were classified: Group 1, healthy newborns with serum total bilirubin (STB) levels that were measured <12 mg/dL; Group 2, newborns with jaundice who were not hospitalized and STB level measured  $\geq 12$  mg/dL and Group 3, newborns with jaundice who were hospitalized for phototherapy and had no identifiable pathologic cause.

## Analysis of cord blood

Umbilical venous blood samples were obtained from all infants immediately after delivery and transferred into an ethylenediaminetetraacetic acid (EDTA)-treated sample bottle. All cord blood samples were analyzed using an automated hematology analyzer (ABX Pentra DX 120, Horiba Ltd., UK) to determine the total white blood cell (WBC) count. The nRBC count was described per 100 WBC by the Wright stain of the blood smear, and also absolute nRBC count per cubic millimeter of cord blood was calculated.

## Statistical analysis

Statistical analyses were performed using SPSS software (version 20.0, SPSS Inc, Chicago, IL, USA). The mean and continuous variables were expressed as mean  $\pm$  standard deviation. Appropriate statistical tests were conducted including Student's T-test to compare mean values, Mann-Whitney U-test to compare nonparametric values and  $\chi^2$ -test to determine the differences between the two groups. p-Values less than 0.05 were accepted as statistically significant.

To determine the specificity of the cord blood nRBC and absolute nRBC counts, receiver operating characteristic (ROC) curve and the associated "area under the curve" (AUC) values were calculated. The AUC values were distributed between 0.5 and 1.0. If a parameter had a value of 0.5, it represented that the parameter had no determinative value, whereas a value of 1.0 represented a highly determinative value. To assess model fit, the Hosmer-Lemeshow test was used. In addition, the sensitivity and specificity of the cutoff scores were determined.

## Results

Overall, 149 eligible newborn infants were enrolled into the study. The mean gestational age was  $38.4 \pm 1.4$  week, the mean birth weight  $3190.8 \pm 433$  g and 76 (51%) babies were male. Sixty-three newborns were delivered vaginally. History of neonatal hyperbilirubinemia in siblings was present in 23 infants.

There was no statistically significant difference for the clinical parameters such as birth weight, gestational age, gender, delivery type and history of neonatal hyperbilirubinemia in siblings (Table 1). The cutoff value with sensitivity and specificity of the first measurement of serum bilirubin in cord blood is 3.00 mg/dL, 86.8% and 62.1%, respectively.

**Table 1:** Clinical characteristics of the study groups.

	Group 1 (n=101)	Group 2 (n=20)	Group 3 (n=28)	p-Value
Gestational age, week <sup>a</sup>	3179 $\pm$ 457	3166 $\pm$ 265	3248 $\pm$ 445	0.89
Birth weight, g <sup>a</sup>	38.5 $\pm$ 1.3	38.2 $\pm$ 1.2	38.3 $\pm$ 1.7	0.49
Gender (male/female)	46/55	10/10	20/8	0.05
Delivery type (normal/cesarean)	45/56	6/14	12/16	0.48
Jaundice in previous sibling	22	5	7	0.9

<sup>a</sup>Data are represented as mean  $\pm$  standard deviation.

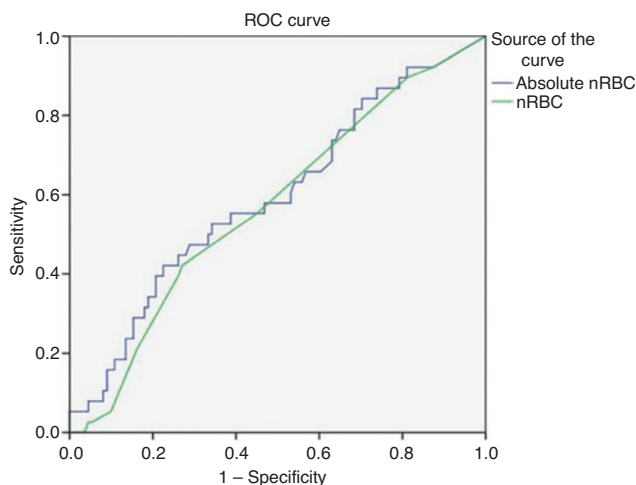
**Table 2:** Complete blood count parameters, nRBC and absolute nRBC counts, and total serum bilirubin levels.<sup>a</sup>

	Group 1 (n=101)	Group 2 (n=20)	Group 3 (n=28)	p-Value
Hb, g/dL	15.8±2.05	15.8±1.9	16.5±2.4	0.32
Htc, %	49.3±6.2	48.4±5.8	50.7±6.6	0.44
WBC (1/mm <sup>3</sup> )	13,658±4897	12,497±3848	13,194±3561	0.64
Thrombocytes (1/mm <sup>3</sup> )	283,217±90,382	256,350±84,514	291,428±66,028	0.30
nRBC count (/100 WBC)	7.9±8.1	10.3±7.9	11.1±6.2	0.01
Absolute nRBC count (1/mm <sup>3</sup> )	964±1044	1696±2551	1790±2764	0.02
TSB in cord blood, mg/dL	1.69±0.39	1.87±0.44	1.95±0.56	0.048
TSB on day 1, mg/dL	2.68±1.84	4.1±2.02	5.5±2.17	<0.001
TSB on day 3, mg/dL	5.09±2.45	9.3±3.5	11.9±2.71	<0.001
TSB on day 7, mg/dL	5.33±2.69	10.3±3.6	–	0.003
TSB on day 15, mg/dL	3.47±2.62	6.74±4.16	–	0.01

<sup>a</sup>Data are represented as mean ± standard deviation. Hb, hemoglobin; Htc, hematocrit; WBC, white blood cell; nRBC, nucleated red blood cell; TSB, total serum bilirubin.

The comparison of cord blood complete blood count parameters, nRBC and absolute nRBC counts, and total serum bilirubin levels are presented in Table 2. Complete blood counts were not statistically different between the groups. Significant statistical differences between the groups were found for nRBC and absolute nRBC counts ( $p=0.01$  and  $0.02$ , respectively). The nRBC count and absolute nRBC counts and total serum bilirubin levels were higher in the group 3 infants.

The AUC calculations from ROC analysis were 0.66 for nRBC count and 0.62 for absolute nRBC count (Figure 1). The sensitivity of the absolute nRBC score was higher than the nRBC count. However, specificity was higher in nRBC count. The negative predictive value was similar whereas the nRBC count has the higher positive predictive value than the absolute nRBC count (Table 3).

**Figure 1:** ROC of the sensitivity and specificity of nRBC and absolute nRBC.**Table 3:** Evaluation of the accuracy of the cord blood nRBC and absolute nRBC counts for predicting neonatal jaundice.

	nRBC	Absolute nRBC
AUC	0.66	0.62
Cutoff value	5.5	406
Sensitivity, % <sup>a</sup>	75 (60.4–86.3)	85.4 (72.2–93.9)
Specificity, % <sup>a</sup>	53.4 (43.2–63.4)	29.7 (21–39.6)
Positive predictive value, % <sup>a</sup>	43.3 (37–49.9)	36.6 (32.7–40.6)
Negative predictive value, % <sup>a</sup>	81.8 (72.7–88.3)	81 (66.9–90)

<sup>a</sup>Values in parenthesis represent 95% confidence interval.

## Discussion

Neonatal jaundice is a common and generally benign condition, occurring in 60%–70% of healthy term infants [11]. However, permanent neurological damage occurs due to bilirubin encephalopathy in severe hyperbilirubinemia. The most important method to prevent bilirubin encephalopathy is to predict the babies who will develop severe neonatal hyperbilirubinemia. Implementation needs to be a system-based approach that allows for individualized care to accommodate the clinician's concerns, informed participation of the family and monitoring of the progression of hyperbilirubinemia of at-risk newborns [12]. To prevent bilirubin encephalopathy, it is recommended to determine the clinical risk factors for severe hyperbilirubinemia and measure bilirubin levels before discharge [13].

As far as we know, our study is the first study to evaluate nRBC levels in newborn infants with jaundice. In this study, nRBC and absolute nRBC counts in cord blood were significantly higher in newborns with jaundice. However,

they seem insufficient to detect the babies who need phototherapy. Our findings suggest that the increase of nRBCs may be a valuable marker to predict the development of neonatal hyperbilirubinemia.

Bilirubin is considered as a toxic compound when it accumulates in high amounts in an organism [14]. *In vivo*, reactive oxygen free radicals are produced during environmental metabolism as well as due to environmental pollutants. The adverse effects of these oxidants on the cell are eliminated by various antioxidants. The disequilibrium between oxidant and antioxidant systems is involved in the pathogenesis of many systemic diseases [15]. Bilirubin is an important antioxidant at physiological limits, but acts as a stronger pro-oxidant at higher levels. Especially in the neonatal period, bilirubin acts as an important cytoprotectant as the antioxidant defense systems of infants do not provide adequate protection against circulating free radicals [16]. Research shows that increased oxidative stress may result in triggering hyperbilirubinemia in neonates, and high serum bilirubin level may protect the cells from oxidative damage [17, 18]. In addition, heme oxygenase may play an important role in the process of oxidative stress. The promoter region of heme oxygenase-1 contains an antioxidant response element. Therefore, the induction of heme oxygenase occurs as a general response to oxidant stress [19–21]. These mechanisms may influence bilirubin production as a protection against oxidative stress by affecting the erythroid series in newborn infants with oxidative stress.

In conclusion, we emphasize that increased nRBC counts in cord blood may be indicative of increased levels of bilirubin in the newborn. This may be due to the toxic effect of bilirubin or increased bilirubin production to reduce oxidative stress. The nRBC count in cord blood may be an effective method for predicting hyperbilirubinemia. Evaluation of nRBC in cord blood is a non-invasive, easy and inexpensive method. Therefore, it can be used as a new marker to guide the physicians for following the infants to reduce the anxieties of the families and to prevent performing unnecessary tests.

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## References

- Maisels MJ, Baltz RD, Bhutani VK, Newman TB, Rosenfeld W, Stevenson DK, et al. Neonatal jaundice and kernicterus. *Pediatrics* 2001;108:763–5.
- Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. *N Engl J Med* 2001;344:581–90.
- Stevenson DK, Dennery PA, Hintz SR. [Understanding newborn jaundice](#). *J Perinatol* 2001;21(Suppl 1):S21–4.
- Curdy EM, Bradley CF. A randomized, controlled evaluation of early postpartum hospital discharge. *Birth* 1990;17:199–204.
- Maisels MJ, Kring E. Length of stay, jaundice and hospital readmission. *Pediatrics* 1998;101:995–8.
- Lee K-S, Perlman M, Ballantyne M. Association between duration of neonatal hospital stay and readmission rate. *J Pediatr* 1995;127:758–66.
- Maisels MJ, Newman TB. Kernicterus in otherwise healthy, breast-fed term newborns. *Pediatrics* 1995;96:730–3.
- Hermansen MC. Nucleated red blood cells in the fetus and newborn. *Arch Dis Child Fetal Neonatal Ed* 2001;84:F211–5.
- Nicolaides KH, Thilaganathan B, Mibashan RS. Cordocentesis in the investigation of fetal erythropoiesis. *Am J Obstet Gynecol* 1989;161:1197–200.
- Leikin E, Verma U, Klein S, Tejani N. Relationship between neonatal nucleated red blood cell counts and hypoxic-ischemic injury. *Obstet Gynecol* 1996;87:439–43.
- American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* 2004;114:297–316.
- Johnson LH, Bhutani VK, Brown AK. System-based approach to management of neonatal jaundice and prevention of kernicterus. *J Pediatr* 2002;140:396–403.
- Keren R, Bhutani VK, Luan X, Nihtianova S, Cnaan A, Schwartz JS. Identifying newborns at risk of significant hyperbilirubinemia: a comparison of two recommended approaches. *Arch Dis Child* 2005;90:415–42.
- Chuniaud L, Dessante M, Chantoux F, Blondeau JP, Francon J, Trivin F. Cytotoxicity of bilirubin for human fibroblasts and rat astrocytes in culture: effect of the value of bilirubin to serum albumin. *Clin Chim Acta* 1996;256:103–14.
- Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987;235:1043–6.
- Bracci R, Buonocore G, Talluri B, Berni S. Neonatal hyperbilirubinemia: evidence for a role of the erythrocyte enzyme activities

- involved in the detoxification of oxygen radicals. *Acta Paediatr Scand* 1988;77:349–56.
17. Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci USA* 2002;99:16093–8.
  18. Turgut M, Basaran O, Cekmen M, Karatas F, Kurt A, Aygun AD. Oxidant and antioxidant levels in preterm newborns with idiopathic hyperbilirubinaemia. *J Paediatr Child Health* 2004;40:633–7.
  19. Dennery PA, Weng YH, Stevenson DK, Yang G. [The biology of bilirubin production](#). *J Perinatol* 2001;21(Suppl 1):S17–20.
  20. Choi AM, Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 1996;15:9–19.
  21. Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. *Cancer Res* 1991;51:974–8.