

Research Article

Correlation of nucleated red blood cells with severity of birth asphyxia and its immediate outcome in term newborns

Pradeep Meena*, Maheshwar Gunawat, Rameshwar L. Suman

Department of Pediatrics, RNT Medical College, Udaipur (Rajasthan), India

Received: 25 April 2016

Accepted: 05 May 2016

*Correspondence:

Dr. Pradeep Meena,

E-mail: drpradeepmeena@ymail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: The severity of birth asphyxia can be predicted by APGAR score and umbilical cord blood pH. The Nucleated Red Blood Cells (NRBCs) in the cord blood at birth emerged as a newer predictor of birth asphyxia. This study was conducted to find the correlation of cord blood Nucleated Red Blood Cell (NRBC) count with the severity of birth asphyxia and its immediate outcome in term newborns.

Methods: An observational hospital based prospective case control study was conducted at inborn NICU, Bal Chikitsalya, RNT Medical College, Udaipur (Raj.) INDIA from March 2015 to September 2015. Total 100 term newborns including 50 as cases (with birth asphyxia) and 50 as controls (normal babies) were enrolled for the study. The cord blood was collected immediately after birth for the measurement of NRBC/100 WBC count and pH value. All the enrolled cases were admitted in NICU and observed for the immediate neurological outcome. The correlation of cord blood NRBC count with APGAR scores, cord blood pH and outcome was analysed statistically.

Results: The mean NRBC (NRBC/100 WBC) count was 17.82 ± 19.55 (range from 0-102) in case group and 1.42 ± 3.26 (range from 0 to 18) in control group ($p < 0.001$). A statistically significant negative correlation of NRBC count was found with severity of birth asphyxia.

Conclusions: A simple, cheap, rapid and non-invasive test of NRBC count from umbilical cord blood correlates well with APGAR score and cord blood pH to predict the severity of birth asphyxia and immediate outcome.

Keywords: APGAR score, Birth asphyxia, Cord blood pH, Nucleated red blood cells, Umbilical cord blood

INTRODUCTION

Asphyxia, derived from the Greek word, a-sphyxos, meaning born without an evident pulse. Asphyxia is most likely complication in a newborn which can occur at the time of birth or in perinatal period and known as Birth Asphyxia/perinatal asphyxia. This is one of the most important causes of fetal distress.¹

Perinatal Asphyxia is a serious problem globally and it is a common cause of neonatal mortality and long-term morbidity. It ranks as a second most common cause of neonatal death after infection accounting for around 30%

mortality worldwide. The data from National Neonatology Forum NNPD Network suggest that perinatal asphyxia contributes to almost 20% of neonatal deaths in India.² Of the 1.2 million neonatal deaths in India every year, 300,000-350,000 infants die due to Perinatal Asphyxia mostly within first 3 days of life.³

A gold standard definition of birth asphyxia does not exist. It is thus appropriate to use the term Perinatal Asphyxia as asphyxia may occur in utero, during the process of labor, at birth or in the postnatal period. World Health Organization (WHO) has defined perinatal asphyxia as a "failure to initiate and sustain breathing at

birth".³ The National Neonatology Forum NNPD Network of India defines moderate asphyxia as slow gasping breathing or an APGAR score of 4-6 at 1 min of age and severe asphyxia as no breathing or an APGAR score of 0-3 at 1 min of age.² American Academy of Pediatrics (AAP) and American College of Obstetrics and Gynaecology (ACOG) proposed that term Perinatal Asphyxia should be reserved to describe a newborn who manifests all of the following features:⁴

- Profound metabolic or mixed acidemia ($\text{pH} < 7.00$) in umbilical cord blood and base deficit of $> 10 \text{ mEq/L}$.
- Persistence of low Apgar scores less than 3 for more than 5 minutes.
- Signs of neonatal neurologic dysfunction (e.g., seizures, encephalopathy, tone abnormalities).
- Evidence of multiple organ involvement (such as that of kidneys, lungs, liver, heart and intestine).

Hypoxic insult to various organs may lead to multi organ failure/dysfunction. Persistent fetal hypoxia leading to Hypoxic-ischemic encephalopathy (HIE), severity of which depends on the duration and timing of hypoxic injury, is an important cause of permanent damage to CNS tissues that may result in neonatal death or manifest later as cerebral palsy or developmental delay. The greatest risk of adverse outcome is seen in infants with severe fetal acidosis ($\text{pH} < 6.7$) (90% death/impairment) and a base deficit $> 25 \text{ mmol/L}$ (72% mortality).⁵

At the time of birth many indicators are there which predicts the severity of asphyxia which includes APGAR score at 1 minute, pH and base deficit in cord blood. The Nucleated RBCs in the cord blood at birth emerged as a newer indicator of severity of Birth Asphyxia.⁶ Considering the hematopoietic response to hypoxia in utero, the elevated NRBC count was investigated as a possible marker of asphyxia in various studies.⁷⁻⁹

The present study was undertaken to evaluate the cord blood NRBC count as a marker of perinatal asphyxia in conjunction with other clinical markers and its ability to predict severity of perinatal asphyxia and its immediate outcome in term newborn.

METHODS

This observational hospital based prospective case control study was carried out at inborn NICU, Department of Pediatrics, RNT Medical College, Udaipur, Rajasthan, INDIA between the period of March 2015 to September 2015. The study was done after approval from ethical committee of our institute. After calculating the sample size by using Epi Info 6 software total 100 term newborns including 50 cases (Asphyxiated) and 50 controls (non asphyxiated) were enrolled, who delivered at Pannadhy Rajkiya Mahila Chikitsalaya, Udaipur (Raj).

Inclusion criteria

Term AGA Newborns with APGAR score < 7 at 1 min of life or normal respiration not established at 1 min after birth or requiring resuscitative measures and willing for consent. The controls were selected as the next term AGA baby delivered after the birth of an asphyxiated neonate.

Exclusion criteria

Preterm (< 37 weeks of gestation), post term babies (≥ 42 weeks of gestation), Rh Incompatibility, Intrauterine growth retarded (IUGR) babies, babies of diabetic and hypertensive (PIH) mothers, babies with history of maternal smoking, history of blood loss in mother, history of chorioamnionitis in mother, babies with severe congenital malformations, babies with Chromosomal abnormalities and or not willing for consent.

Immediately after birth of the baby, 1 ml of blood was collected in heparinised syringe from doubly clamped segment of umbilical cord for Arterial Blood Gas Analysis (ABG) and another 2 ml blood collected in ethylenediaminetetraacetic acid (EDTA) vial for routine haematological investigations. Simultaneously a thin blood film was prepared on a glass slide, which was dried and stained with Leishman's stain. The slide was examined with oil immersion lens for Nucleated RBCs and leucocytes. NRBC's were counted till 500 white blood cells (WBCs) and then was reported as NRBC/100 WBCs.

Normal healthy babies (Controls) were kept with mothers and the asphyxiated babies (cases) were shifted to neonate intensive care unit (NICU) after resuscitation for further monitoring and screening for development of Hypoxic Ischemic Encephalopathy (HIE) and staging done as per Sarnat and Sarnat staging system.¹⁰ The cases were observed for immediate outcome for first 24 hours of life. Daily Progress of cases was recorded. The HIE and other complication detected during hospital stay were managed as per standard protocols. All the cases were followed up till the discharge or death. The correlation of cord blood NRBC count with APGAR score, cord blood pH, HIE Staging and final outcome was evaluated and analysed statistically.

RESULTS

Total 100 newborns were enrolled for the study including 50 asphyxiated newborns as cases and 50 non asphyxiated as controls. Various clinical and laboratory variables were studied and analyzed in both the groups. All the clinical parameters were comparable in both the groups except mode of delivery, type of amniotic fluid and APGAR score. The mode of delivery was LSCS in 50% cases and 14% controls whereas NVD was mode of delivery in 50% cases and 86% controls. The amniotic fluid was meconium stained in 48% of case and only 10%

in controls. The mean APGAR at 1 min and 5 min in the case group was 3.76 ± 1.04 and 5.82 ± 1.88 and in control group it was 8.20 ± 0.64 and 9.28 ± 0.45 (Table 1).

Similarly all the laboratory variables (Table 2) were also comparable in both the groups except WBC Count, NRBC count, s. potassium, cord blood pH and Bicarbonates. The mean WBC count was 16.78 ± 6.32 in

case group and 10.09 ± 3.00 in control groups. The Mean NRBC Count in cases was 17.82 ± 19.55 and in the controls it was 1.42 ± 3.26 . The mean of S. Potassium value in cases was 4.76 ± 0.57 and in controls 4.13 ± 0.66 . Mean cord blood pH and bicarbonate were 7.18 ± 0.14 and 15.70 ± 3.68 in the case group; 7.32 ± 0.05 and 20.15 ± 1.05 in controls.

Table 1: Clinical variables in cases and controls.

| Parameters | Cases (n=50) | Control (n=50) | p value |
|---|------------------|------------------|---------|
| Male | 27 (54%) | 25 (50%) | 0.68 |
| Female | 23 (46%) | 25 (50%) | 0.68 |
| Maternal age (yrs) | 23.76 ± 2.75 | 23.76 ± 1.80 | 1.00 |
| Hb of mother (gm/dl) | 10.83 ± 0.96 | 11.05 ± 0.87 | 0.23 |
| GA (weeks) | 39.20 ± 1.05 | 39.80 ± 0.51 | >0.05 |
| Birth weight (Kg) | 2.92 ± 0.33 | 2.92 ± 0.27 | 1.00 |
| Mode of delivery (NVD/LSCS) | 25/25 (50%/50%) | 43/07 (86%/14%) | <0.001 |
| Presentation (vertex/breech) | 46/04 (92%/08%) | 45/05 (90%/10%) | 0.75 |
| Type of amniotic fluid (clear/meconium stained) | 26/24 (52%/48%) | 45/05 (90%/10%) | <0.001 |
| Mean APGAR at 1min | 3.76 ± 1.04 | 8.20 ± 0.64 | <0.001 |
| Mean APGAR at 5 min | 5.82 ± 1.88 | 9.28 ± 0.45 | <0.001 |

Table 2: Laboratory variables in cases and controls.

| Lab parameter | Cases | Control | p value |
|----------------------------------|-------------------|-------------------|---------|
| Hematological | | | |
| Baby Hb (gm/dl) | 16.81 ± 1.68 | 16.50 ± 1.06 | 0.27 |
| WBC Count ($10^3/\text{mm}^3$) | 16.78 ± 6.32 | 10.09 ± 3.00 | <0.001 |
| TRBC ($10^6/\mu\text{L}$) | 5.73 ± 0.60 | 5.81 ± 0.40 | 0.43 |
| Mean NRBC Count | 17.82 ± 19.55 | 1.42 ± 3.26 | <0.001 |
| S. electrolytes | | | |
| Na^+ (mEq/L) | 134.11 ± 5.91 | 134.94 ± 4.39 | 0.42 |
| K^+ (mEq/L) | 4.76 ± 0.57 | 4.13 ± 0.66 | <0.001 |
| Ca^{++} (mg/dl) | 9.72 ± 0.83 | 9.54 ± 0.60 | 0.21 |
| Cord blood ABG | | | |
| pH | 7.18 ± 0.14 | 7.32 ± 0.05 | <0.001 |
| HCO_3 (mmol/L) | 15.70 ± 3.68 | 20.15 ± 1.05 | <0.001 |
| p CO_2 (mmHg) | 46.72 ± 11.22 | 41.50 ± 6.19 | 0.005 |
| p O_2 (mmHg) | 63.52 ± 16.69 | 64.62 ± 12.72 | 0.71 |

In the distribution of NRBC count in cases and controls (Table 3), 42% newborns in case group and 98% newborns in control group were falling in the <11 NRBC/100WBC range group. Remaining 58% of cases and 2% of controls were falling in ≥ 11 NRBC/100 WBC range group.

We analysed the APGAR scores, cord blood pH and cord blood NRBC count in relation to HIE staging (Table 4). The mean APGAR at 1 min and 5 min, mean cord blood pH and mean NRBC count in the babies without HIE

were 4.53 ± 0.70 and 7.37 ± 0.96 , 7.28 ± 0.08 and 4.05 ± 4.77 , respectively. Whereas the mean APGAR at 1 min and 5 min, mean cord blood pH and mean NRBC count in the babies with HIE-I were 4.40 ± 0.89 and 6.80 ± 0.45 , 7.21 ± 0.11 and 12 ± 7.87 ; HIE-II 3.70 ± 0.67 and 6.0 ± 1.25 , 7.20 ± 0.06 and 15.2 ± 3.90 ; HIE-III 2.69 ± 0.60 and 3.56 ± 0.81 , 7.03 ± 0.11 and 37.63 ± 22.81 .

Table 3: Range of distribution of NRBC count in cases and controls.

| NRBC counts | Cases | Controls |
|-------------|----------|----------|
| <11 | 21 (42%) | 49 (98%) |
| 11-20 | 14 (28%) | 1 (2%) |
| 21-30 | 5 (10%) | - |
| 31-40 | 6 (12%) | - |
| 41-50 | 1 (2%) | - |
| >50 | 3 (6%) | - |
| Grand Total | 50 | 50 |

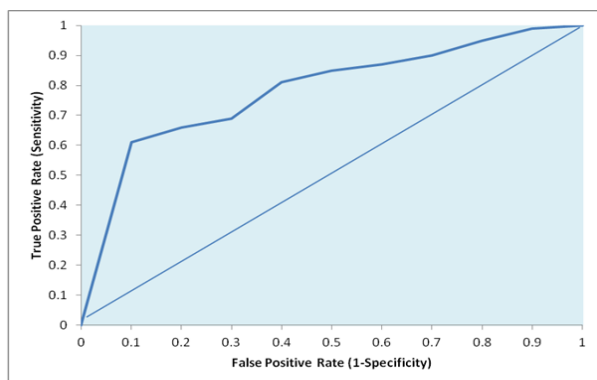
All the cases were observed for immediate outcome during the first 24 hours of life. Out of 50 cases, 21% were neurologically normal, 72% abnormal and one patient died within first 24 hours. The mean cord blood NRBC count and pH in neurologically normal babies were 3.09 and 7.27, whereas in neurologically abnormal babies mean NRBC count and cord blood pH were 19.87 and 7.16. In one baby who died the NRBC count and cord blood pH were 102 and 6.98, respectively (Table 5).

Table 4: APGAR score, cord blood pH and NRBC count according to HIE staging.

| Birth asphyxia | HIE staging | Mean APGAR 1 min | Mean APGAR 5 min | Mean cord blood pH | Mean cord blood NRBC |
|----------------|-------------|------------------|------------------|--------------------|----------------------|
| Without HIE | NO | 4.53±0.70 | 7.37±0.96 | 7.28±0.08 | 4.05±4.77 |
| With HIE | 1 | 4.40±0.89 | 6.80±0.45 | 7.21±0.11 | 12±7.87 |
| | 2 | 3.70±0.67 | 6.00±1.25 | 7.203±0.06 | 15.20±3.90 |
| | 3 | 2.69±0.60 | 3.56±0.81 | 7.03±0.11 | 37.63±22.81 |

The relation of APGAR score at 1 min, cord blood pH and NRBC count with final outcome was also analysed. The mean values of these parameters in discharged group (n=34) were 4.26, 7.25, 8.50, respectively (Table 6). Amongst the cases who died (n=15) mean values of APGAR at 1 min, cord blood pH and NRBC count were 2.73, 7.04 and 38.93. There was a significant negative correlation coefficient of NRBC count with APGAR at 1 min, APGAR 5 min and cord blood pH at birth (Table 7).

ROC curve (Figure 1) was plotted for the cut off value of NRBC count (NRBC/100 WBC) of 10 for predicting the asphyxia with umbilical cord pH value of <7.20. The sensitivity and specificity were 66.67% and 90.00%, respectively (Figure 1). The positive and negative predictive values were 90.91% and 64.29% respectively (Table 6).

**Figure 1: Receiver operating curve (ROC) to discriminate the sensitivity and specificity of NRBC count in diagnosis of birth asphyxia.****Table 5: NRBC count and cord blood pH with immediate outcome.**

| Immediate outcome | Cases | Mean NRBC count | Mean cord blood pH |
|-------------------|----------|-----------------|--------------------|
| Normal | 11 (21%) | 3.09 | 7.27 |
| Abnormal | 38 (72%) | 19.87 | 7.16 |
| Death | 1 (2%) | 102 | 6.98 |

Table 6: APGAR score at 1 min, cord blood pH and NRBC count with final outcome.

| Final outcome | Cases | Mean APGAR 1 min | Mean cord blood pH | Mean NRBC count |
|---------------|----------|------------------|--------------------|-----------------|
| Discharge | 34 (68%) | 4.26 | 7.25 | 8.50 |
| LAMA | 1 (2%) | 2 | 6.91 | 18 |
| Death | 15 (30%) | 2.73 | 7.04 | 38.93 |

Table 7: Correlation coefficient of NRBCs with APGAR at 1 min, APGAR at 5 min and cord blood pH.

| Parameter | r | p |
|----------------|--------|--------|
| APGAR at 1 min | -0.633 | <0.001 |
| APGAR at 5 min | -0.666 | <0.001 |
| Cord blood pH | -0.624 | <0.001 |

Table 8: Sensitivity, specificity and predictive value of NRBC count.

| Cord blood pH | NRBC/100 WBC | | |
|--|--------------|-----|-------|
| | >10 | <10 | Total |
| <7.20 | 20 | 2 | 22 |
| ≥7.21 | 10 | 18 | 28 |
| Total | 30 | 20 | 50 |
| Sensitivity, specificity and predictive value | | | |
| Sensitivity | 66.67% | | |
| Specificity | 90.00% | | |
| Positive predictive value | 90.91% | | |
| Negative predictive value | 64.29% | | |

DISCUSSION

The Nucleated RBCs are the immature cells of erythropoietic series. Their number increases in circulation in response to hypoxia as a result of increased erythropoietin secretion. The cord blood NRBC count at the time of birth is claimed to be a good predictor of Birth Asphyxia.¹¹⁻¹⁴ In the present study, attempt has been made to evaluate the relation of cord blood NRBC Count in predicting birth asphyxia and its immediate outcome.

In our study, the mean NRBC count was significantly higher in case group as compared to controls, which supports the previously suggested view that NRBC's are released in stressful condition of birth asphyxia due to hypoxia. It was also noted that the NRBC count was more in cases which delivered by LSCS and or having meconium stained amniotic fluid. The relation between HIE staging, APGAR score, cord blood pH and NRBC count was analysed in the cases. We noted that as the HIE severity increases, the values of mean APGAR score and cord blood pH decreases whereas the Mean NRBC count increases. The similar observations were also noted in previous studies.¹¹⁻¹⁷

We also evaluated the relation between patient outcome, Cord blood pH and NRBC Count. The mean cord blood pH was low in cases having unfavorable immediate and final outcome whereas mean NRBC count was on higher side in these cases.

In our study we found a significant negative correlation coefficient of NRBC count with APGAR score and cord blood pH. Colaco et al also observed a negative correlation coefficient between these.¹⁴ This shows that as severity of birth Asphyxia increases the APGAR score and pH decreases but NRBC count increases.

In our study the NRBC count was found to be a sensitive (Sensitivity: 66.6%) and specific (Specificity: 90%) indicator to predict the birth asphyxia. Similar observation also noted by Colaco et al¹⁴ and Mohanty et al¹⁶.

We concluded that the NRBC count in cord blood at birth can be considered as a good marker of perinatal asphyxia. A simple, cheap, rapid and non-invasive test of cord blood NRBC count provides valuable information about the well-being of the newborn at birth and it correlates well with APGAR score and cord blood pH. The NRBC count can be used as a simple tool to predict the severity and immediate outcome of birth asphyxia in a term newborns in conditions where APGAR score and or ABG machine with expertise not available.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

- Steer PJ, Danielian P. Fetal distress in labour. In: James, Steer, Weiner, Gonik. High Risk Pregnancy. 3rd edition. WB Saunders; 2005:1450-1471.
- National Neonatal and Perinatal Database Report. 2002-2003:1-58. Available at http://www.newbornwhocc.org/pdf/nnpd_report_2002-03.PDF. Accessed on 02 April 2016.
- World Health Organization. Neonatal and perinatal mortality; country, regional and global estimates, 2004; WHO, Geneva. 2006;1-25.
- American Academy of Paediatrics, American College of Obstetrician and Gynaecologists. In: Guidelines for Perinatal Care. Gilstrap LC, Oh W (Eds.) 5th Ed. Elk Grove Village III. American Academy of Pediatrics. 2002:187.
- Namasivayam A, Waldemar A C. Hypoxic ischemic encephalopathy. In: Kliegman RM, Stanton BF, St Geme III JW, Schor NF, eds. Nelson Text Book of Pediatrics. 20th ed. Elsevier Saunders Philadelphia; 2015:838-842.
- Green DW, Hendon B, Mimouni FB. Nucleated erythrocytes and intraventricular hemorrhage in preterm neonates. Pediatrics. 1995;96(3):475-8.
- Forestier F, DaVos F, Catherine N, Renard M, Andreux JP. Developmental hematopoiesis in normal human fetal blood. Blood. 1991;77:2360-3.
- Ghosh B, Mittal S, Kumar S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. Int J Gynaecol Obstet. 2003;81(3):267-71.
- Lippman HS. Morphologic and quantitative study of blood corpuscles in the newborn period. American J Dis Children. 1924;27:473-515.
- Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol. 1976;33(10):696-705.
- Hermansen MC. Nucleated red blood cells In the fetus and newborn. Arch Dis Child Fetal Neonat Ed. 2001;84:211-5.

12. Phelan JP, Ahn MO, Korst LM, Martin GI. Nucleated red blood cells: a marker for fetal asphyxia? *Am J Obstet Gynecol*. 1995;173:1380-4.
13. Hanlon-Lundberg KM, Kirby RS. Nucleated red blood cells as a marker of acidemia in term neonates. *Am J Obstet Gynecol*. 1999;181:196-201.
14. Colaco SM, Ahmed M, Kshirsagar VY, Bajpai R. Study of nucleated red blood cell counts in asphyxiated newborns and the fetal outcome. *Int J Clin Pediatr*. 2014;3(3):79-85.
15. Ferns SJ, Bhat BV, Basu D. Value of nucleated red blood cells in predicting severity and outcome of perinatal asphyxia. *Indian J Pathol Microbiol*. 2004;47(4):503-5.
16. Mohanty AK, Das L, Pradhan S, Meher B, Beriha SS. Cord blood nucleated RBC as a predictor of perinatal asphyxia, severity and outcome. *Indian J Clin Pract*. 2014;24(10): 983-6.
17. Boskabadi H, Afshari JT, Ghayour-Mobarhan M, Maamouri GH, Shakeri MT, Sahebkar A. Association between serum interleukin-6 levels and severity of perinatal asphyxia. *Asian Biomed*. 2010;4:79-85.

Cite this article as: Meena P, Gunawat M, Suman RL. Correlation of nucleated red blood cells with severity of birth asphyxia and its immediate outcome in term newborns. *Int J Contemp Pediatr* 2016;3: 841-6.