A Clinical Study for Prediction of Perinatal Asphyxia using Nucleated Red Blood Cells as an Indicator in Umbilical Cord Blood of Newborns

Jyoti Biswas[1], Alka Bhambri[2], Vedika A. Bhat[3]

ABSTRACT

Introduction: Perinatal asphyxia has become the leading cause of death for newborns. Since the parameters that are being routinely used as a predictor for perinatal asphyxia such as thick meconium stained liquor, non-reassuring fetal heart patterns, low Apgar scores, fetal or cord blood pH do not show consistent correlation with fetal acidosis but nucleated red blood cell counts in umbilical venous blood of neonates has been reported as a possible marker of perinatal asphyxia. The number of nucleated red blood cells (nRBCs)/100 white blood cells is variable but is rarely greater than 10 in normal neonates. The aim of this study was to study the using of nucleated red blood cells in umbilical cord blood of newborns as an indicator for perinatal asphyxia.

Material and methods: The study comprised of intramural term neonates with and without asphyxia. It was a case control study conducted in the Department of Pediatrics in collaboration with Department of Obstetrics and Gynaecology, Rohilkhand Medical College and hospital, Bareilly from November 2017 to October 2018. There were 50 asphyxiated newborns and 50 healthy newborns in the study. The mean levels of nRBCs in cord blood were significantly higher (p value<0.001) in the asphyxial group (54.06+-22.42) as compared to control group (10.32+-5.86).

Results: Nucleated red blood cells were found to be strongly associated with perinatal asphyxia. The mean values of cord blood nRBCs in cases were 54.06+-22.42 and in control group were 10.32+-5.86. The p value being <0.001 which was statistically significant indicating the correlation.

Conclusions: Therefore it was concluded that nucleated red blood cells in umbilical cord blood of newborns can be used as an effective test for prediction of perinatal asphyxia.

Keywords: Nucleated Red Blood Cells, Newborns, Perinatal Asphyxia, Umbilical Cord Blood, Umbilical Cord pH

INTRODUCTION

Asphyxia is a word derived from the Greek word aspyxos, meaning born without an evident pulse. Asphyxia present in perinatal period is one of the most important causes of fetal distress. It refers to a condition of impaired gas exchange that leads, if persistent, to fetal hypoxemia and hypercarbia. A gold standard definition of birth asphyxia does not exist, therefore it is appropriate to use the term perinatal asphyxia as asphyxia which may occur in utero, during the process of labor, at birth or in the post-natal period.

The data from National Neonatology Forum NNPD Network suggests that Perinatal Asphyxia contributes to almost 20% of neonatal deaths in India.[1] Of the 1.2 million neonatal deaths in India every year, 300,000 - 350,000 infants die due to Perinatal Asphyxia mostly within the first 3 days of life.[2]

Nucleated red blood cells (nRBCs) can be easily found in neonatal blood and its count in neonatal umbilical venous blood has been distinguished as a marker of perinatal asphyxia.[3] The number of nRBCs/100 WBC is variable but is rarely greater than 10 in normal neonates.[3-6] There is a need for a reliable marker to predict the course of hospital stay and short term prognosis in term newborns with perinatal asphyxia. It has been observed by several studies that chronic or acute hypoxia leads to increased nucleated RBC count (nRBC count) in a neonate.[7]

The hypoxic event induces a compensatory response in the form of exaggerated erythropoiesis, resulting in the release of immature red blood cells into the fetal circulation. Since the present indices of asphyxia are unhelpful in the diagnosis and prediction of the severity of asphyxia, we wished to investigate the relationship between the absolute nRBC count, nRBC count/100 WBC, the severity of perinatal asphyxia and clinical outcomes in neonates.

The aim of this study was to study the using of nucleated red blood cells in umbilical cord blood of newborns as an indicator for perinatal asphyxia.

MATERIAL AND METHODS

This was a prospective study conducted in the Department of Pediatrics in collaboration with Department of Obstetrics & Gynaecology, Rohilkhand Medical college & Hospital, Bareilly from 1st November 2017 to 31st October 2018. The study was approved by ethical committee of Rohilkhand Medical college & Hospital, Bareilly. A total of 100 babies born at Rohilkhand Medical College and Hospital were enrolled in the study out of which 50 were asphyxiated newborns & 50 were term healthy newborns. An informed written consent from parents was taken.

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Inclusion criteria

1) Signs of fetal distress (heart rate of less than 100 beats per minute, late decelerations, or/and no heart rate variability)
2) Thick, meconium stained amniotic fluid & respiratory distress, hypotonia, or bradycardia.
3) Apgar score of 4 or less at one minute or 6 or less at five minutes.
4) A need for resuscitation for more than 1 minute with positive pressure ventilation and oxygen immediately after birth.
5) Blood pH value of less than 7.20 or a base deficit of at least 12 mmol/L within the first hour after birth.
6) Term neonates with perinatal asphyxia with 5 minute Apgar score less than 7/10.

Exclusion criteria

1) Preterm (<37 weeks of gestation), post term babies (>=42 weeks of gestation).
2) Rh Incompatibility
3) Intrauterine growth retardation (IUGR) babies
4) Babies of diabetic & hypertensive (PIH) mothers
5) Babies with history of maternal smoking
6) History of blood loss in mothers.
7) History of chorioamnionitis in mothers
8) Babies with severe congenital malformations
9) Babies with chromosomal anomalies
10) Those not willing for consent for the study.

Two samples of cord blood were collected immediately after clamping and cutting the umbilical cord. One sample of umbilical cord blood in a EDTA vial was drawn for the purpose of estimation of pH. A second sample of mixed cord blood was taken in a EDTA coated bottle from BD vacutainer system for the purpose of making smears which were stained with Leishmann's stain and findings were checked by a hemato-pathologist. Other details of antenatal and intrapartum events were recorded in predesigned format.

STATISTICAL ANALYSIS

The data were entered on a Microsoft Excel spread sheet and imported into Statistical Package for Social Sciences (SPSS) version 22 for statistical analysis. Data was presented in mean and standard deviation. Independent t-test was performed to find significant difference in different variables between the two groups. A P-value less than 0.05 was considered statistically significant.

RESULTS

We recruited 100 neonates (50 each of cases and controls) in present study. There was statistically no significant difference in maternal age, birth weight, parity of mothers & hematological parameters (Hb, WBC count, RBC count, PCV, MCH, MCHC) of cases and control groups.

In our study the mean values of cord blood nRBCs in cases were 54.06+-22.42 and in control group were 10.32+-5.86. The t value was 13.39 and p value was <0.001 which was statistically significant (table-1).

Our study also showed statistical significance in terms of cord pH in neonates relating its relevance with occurrence of perinatal asphyxia. It also shows correlation with presence of nRBCs in asphyxiated newborns (fig-1,2, table-2).

Upon statistical analysis, it was found that cord blood nRBCs were significantly elevated in asphyxiated group babies as compared to control group. Since cord pH has been used as a predictor for perinatal asphyxia, in our study we found asphyxiated newborns with low cord pH and perinatal

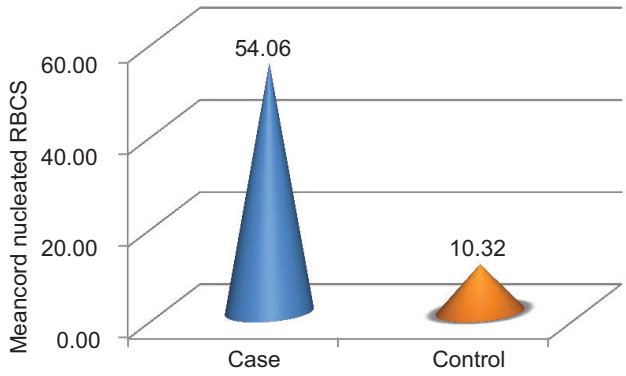

Figure-1: CORD nucleated RBCS

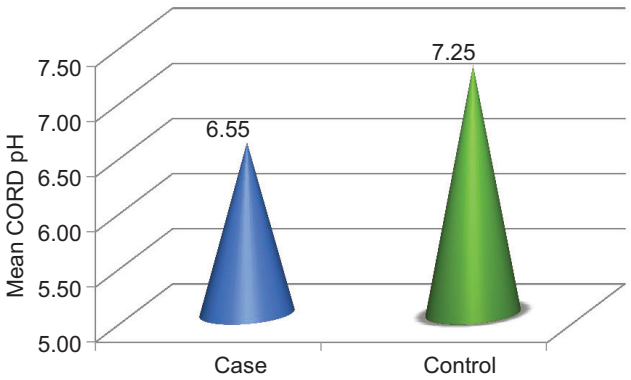

Figure-2: CORD pH

	Case	Control	t-value	P-value
	Mean ± S.D	Mean ± S.D		
Cord nucleated RBCS	54.06 ± 22.42	10.32 ± 5.86	13.39	<0.001*

Table-1: Cord nucleated RBCS

	Case	Control	t-value	P-value
	Mean ± S.D	Mean ± S.D		
CORD pH	6.55 ± 0.24	7.25 ± 0.12	18.93	<0.001*

Table-2: CORD pH

asphyxia had significantly elevated levels of nRBCs. Hence nRBCs could be considered as a reliable predictor of perinatal asphyxia and has significant correlation with cord pH. Therefore we finally concluded that cord blood nRBCs is a good predictor of perinatal asphyxia as per the above analysis.

DISCUSSION

Early recognition of severity of birth asphyxia is important as early institution of therapeutic measures in severe hypoxic ischemic encephalopathy has resulted in better neurological outcome.[8] One of the common finding in term neonates is presence of nucleated RBCs after birth in umbilical cord blood. Their normal values are usually less than 10/ 100 WBCs and are cleared from neonatal blood during the initial week after birth.[9] Perinatal asphyxia is one of the causes for increase in nRBC count in term neonates. While the maternal age, parity, birth weight and hematological parameters of newborns did not significantly vary among cases and controls, we found cord pH, need for positive pressure ventilation>1minute, APGAR scores at 1 minute to be significantly associated to occurrence of birth asphyxia. Also, newborns with birth asphyxia and higher number of nucleated RBCs had significantly poor APGAR score at 1 minute when compared to normal neonates. Korst et al[10], in his study compared nucleated red blood cell count of 153 term neurologically impaired neonates with cord blood nucleated red blood cells of 83 term normal babies. They concluded that nucleated red blood cells identify the presence of fetal asphyxia and distinct nucleated red blood cell patterns were observed that relate to the timing of fetal injury. It may assist in determining the timing of fetal neurologic injury.

Ghosh B et al[11] did a study to determine the normal level of nucleated red blood cells per 100 white blood cells in cord blood of term non asphyxiated newborns and to investigate the variations in nRBC counts in perinatal asphyxia. They concluded that the level of nRBCs per 100 WBC correlates both with acute and chronic antepartum asphyxia and can be used as a reliable variable to predict early neonatal outcome.

Ferns SJ et al[12] did a prospective control study to determine the predictive value in short term outcome and the value of nucleated RBCs in assessing the severity of perinatal asphyxia. They concluded that nRBC count at birth was a useful predictor of severity and short-term outcome of perinatal asphyxia.

Phelan et al[13] determined whether neonatal lymphocyte or nucleated red blood cell (nRBC) counts can be used to date fetal neurologic injury. Term infants with hypoxic ischemic encephalopathy and permanent neurologic impairment were divided into two groups: infants with preadmission injury, manifested by a nonreactive fetal heart rate (FHR) pattern from admission until delivery; and infants with acute injury, manifested by a normal FHR pattern followed by a sudden prolonged FHR deceleration. Lymphocyte and nucleated RBC values were compared with published high normal counts for normal neonates: 8000 lymphocytes/mm3 and 2000 nucleated RBCs/mm3. The study population consisted of 101 neonates. In the first hours of life, lymphocyte counts were elevated among injured newborns, and then the counts rapidly normalized. Brain-injured neonates were 25 times more likely to have a lymphocyte count greater than 8000 than were normal neonates (54 [62%] of 87 versus 6 [7%] of 84; odds ratio 25.5; 95% confidence interval 8.8, 80.1; P < .001). The mean lymphocyte count tended to be higher in the preadmission-injury group than in the acute-injury group. In comparison, nucleated RBC values were not correlated as strongly with neonatal hours of life; nucleated RBC counts tended to be higher and persist longer among neonates with preadmission injury than among those with acute injury. They concluded that compared with normal levels, both lymphocyte and nucleated RBC counts were elevated among neonates with asphyxial injury. Both counts appear to be more elevated and to remain elevated longer in newborns with preadmission injury than in infants with acute injury. However, the rapid normalization of lymphocyte counts in these injured neonates limits the clinical usefulness of these counts after the first several hours of life.

Perri T et al[14] did a study to evaluate fetal nRBCs in prolonged pregnancies (beyond 287 days). They collected umbilical cord blood at delivery from 75 prolonged pregnancies. 150 term deliveries served as controls. Nucleated red blood cell counts were expressed per 100 white blood cells (WBC) with umbilical arterial pH. The median nRBCs per 100 WBCs in prolonged pregnancy was not significantly elevated over the term values (median 3, range 0 - 35 median of 3, range 0 - 34, respectively). A statistically significant (p value <0.05) association between elevated nRBC count and low arterial blood pH was shown in their study, our study too showed similar results.

CONCLUSION

The present study concludes that simple estimation of nRBCs in the cord blood can help us distinguish an asphyxiated from a non-asphyxiated term neonate rapidly. In resource-poor countries, nRBC counts can be a useful part for the evaluation of perinatal asphyxia where facilities for pH sampling are not available and can serve as a reliable, inexpensive and easily available marker of perinatal asphyxia.

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