Research Paper
Cord Blood NRBC Count : A Novel Predictor of Birth Asphyxia in Full Term Newborns

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Abstract
Objective: *To evaluate the role of Cord Blood Nucleated Red Blood Cell (NRBC) Count as a predictor of the Birth Asphyxia in full term newborns.*
Methods: *A hospital based prospective observational case control study was carried out at SNCU, Department of Pediatrics, RNT Medical College, Udaipur (Raj.) from March 2015 to September 2015. Total 100 full term newborns including 50 asphyxiated (cases) and 50 non asphyxiated (controls) were enrolled for the study. The umbilical cord blood was collected at birth for measurement of NRBC count (NRBC/100 WBC) and for Arterial Blood Gas Analysis. All the cases were admitted in SNCU for observation for development of HIE. The relation of cord blood NRBC count with APGAR scores and cord blood pH 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. The sensitivity and specificity of Nucleated RBCs to predict the Birth Asphyxia with cord blood pH <7.2 were 66.67% and 90%, respectively.*
Conclusion: *The Cord Blood NRBC Count is a sensitive predictor of Birth Asphyxia. It is a simple, cheap, rapid and non-invasive test from the umbilical cord blood which provides the valuable information about the neonatal wellbeing at birth and it correlates well with APGAR score and cord blood pH to predict the severity of Birth Asphyxia.*
Keywords: *APGAR Score, Hypoxic Ischemic Encephalopathy, Nucleated Red Blood Cells, Perinatal/Birth Asphyxia, Umbilical Cord Blood pH.*

I. Introduction

Birth Asphyxia is a serious global problem in newborns and it is a common cause of neonatal mortality and morbidity in the form of long term neurological sequele. 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 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 first 3 days of life [2].

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" [2]. 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 [1].

Hypoxia can cause damage to almost every tissue and organ of the body. Hypoxic insult to various organs may lead to multi organ failure / dysfunction. Persistent fetal hypoxia leads to development of Hypoxic-Ischemic Encephalopathy (HIE) and severity depends on the duration and timing of hypoxic injury. HIE is an important cause of permanent damage to CNS and it may resulting either neonatal death or manifesting later as cerebral palsy or developmental delay. The greatest risk of adverse outcome is seen in infants with severe fetal acidosis (pH <6.7) (90% death/impairment) and a base deficit >25 mmol/L (72% mortality) [3].

At birth, the severity of asphyxia is indicated by APGAR score, pH and base deficit in cord blood. During the recent years, cord blood Nucleated RBCs at birth emerged as a newer indicator of severity of Birth Asphyxia [4]. Considering the hematopoietic response to hypoxia in utero, the elevated NRBC count was investigated as a possible marker of asphyxia in various studies [5,6,7].

The present study was carried out to evaluate the role of cord blood NRBC count as a predictor of perinatal asphyxia in full term newborns.

II.   Material And Methods

This was a hospital based prospective observational case control study which was carried out at SNCU, Department of Pediatrics, RNT Medical College, Udaipur, Rajasthan, between the period of March 2015 to September 2015. The study was started after proper approval from Institutional Ethical Committee of RNT Medical College, Udaipur. The minimum sample size for the study was calculated by using Epi Info 6 software. Total 100 term newborns including 50 cases (Asphyxiated) and 50 controls (Non-Asphyxiated), who delivered at Pannadhay Rajkiya Mahila Chikitsalaya, Udaipur (Raj.), were enrolled for the study. The *Inclusion Criteria* for the case group: Full 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 healthy 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 for the study.

Immediately after the 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 EDTA vial for routine haematological investigations. Simultaneously thin blood film was prepared on the glass slide, which was dried and stained with Leishman's stain. The slide was examined under oil immersion lens for Nucleated RBCs and WBCs. The NRBCs were counted till 500 white blood cells (WBCs) and then was reported as NRBC/100 WBCs.

The normal healthy babies (Controls) were kept with mothers and the asphyxiated babies (cases) were shifted to SNCU 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]. Daily progress of cases was recorded. The HIE and other complication detected during hospital stay were managed as per standard protocols. The relation of cord blood NRBC count with APGAR score, cord blood pH and HIE Staging was evaluated and analysed statistically.

III.   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 parameters were studied and analysed in both the groups. *Table 1* shows the various clinical parameters in the cases and controls. All the parameters are 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, respectively and in control group it was 8.20±0.64 and 9.28±0.45, respectively.

Table 1:- Clinical Parameters in Cases and Controls

	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%/4%)	<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

Similarly all the laboratory parameters (*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 2:- Laboratory Parameters in Cases and Controls

Lab parameter	Cases (n=50)	Control (n=50)	p Value
Hematological -			
Baby Hb (gm/dl)	16.81 ± 1.68	16.50 ± 1.06	0.27
WBC Count ($10^3/mm^3$)	16.78 ± 6.32	10.09 ± 3.00	<0.001
TRBC(10^6/μL)	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
HCO3 (mmol/L)	15.70 ± 3.68	20.15 ± 1.05	<0.001
pCO2 (mmHg)	46.72 ± 11.22	41.50 ± 6.19	0.005
pO2 (mmHg)	63.52 ± 16.69	64.62 ± 12.72	0.71

Table 3:- Range Of Distribution of NRBC Count In Cases and Controls

NRBC Counts	Cases	Controls
<11	21(42%)	49(98%)
≥11	29 (58%)	1(2%)

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.

Table 4:- APGAR Score, Cord Blood pH and NRBC Count according to HIE Staging

Birth Asphyxia	HIE Staging	Mean APGAR score at 1 min	Mean APGAR score at 5 min	Mean Cord blood pH	Mean Cord Blood NRBC Count
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±o.11	37.63±22.81

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±o.11 and 37.63±22.81.

Table 5:- Correlation Coefficient of NRBC Count 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 6:- 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	66.67%
Specificity	90.00%
Positive Predictive value	90.91%
Negative Predictive value	64.29%

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 5).***

ROC curve (Fig.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 (***Fig.1)***. The positive and negative predictive values were 90.91% and 64.29% respectively (***Table 6).***
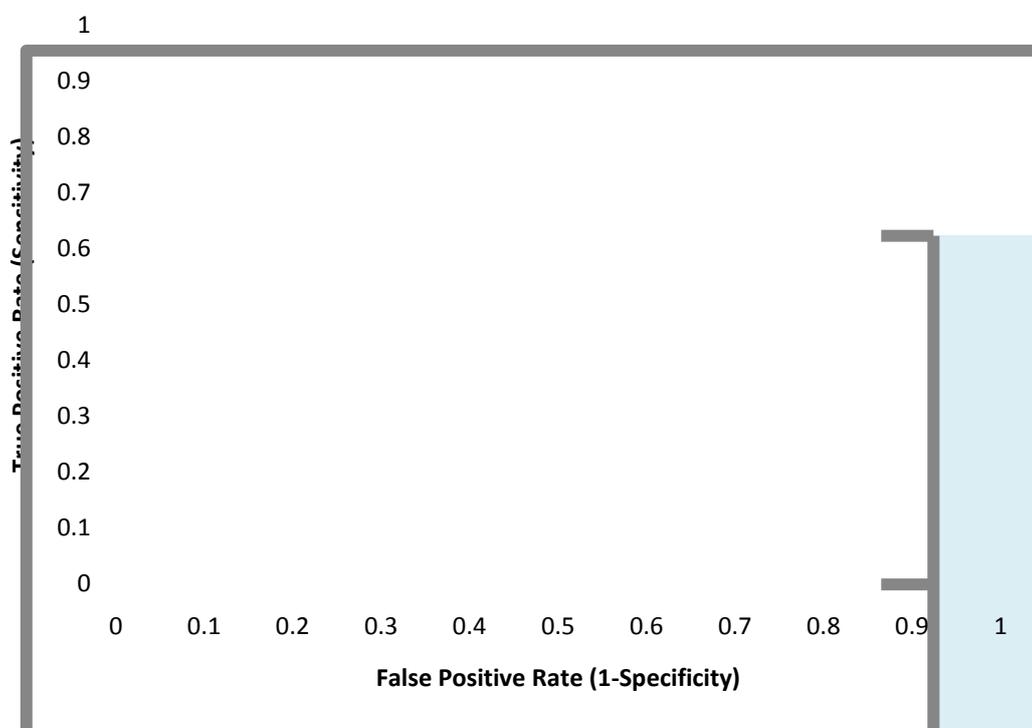
Fig. 1:- Receiver Operating Curve (ROC) to discriminate the Sensitivity and Specificity of NRBC Count in predicting of Birth Asphyxia

IV. Discussion

The Nucleated RBCs are the immature cells of erythropoietic series. They are released prematurely in the circulation in response to hypoxia as a result of increased erythropoietin secretion. The increased NRBC count in the umbilical cord at birth is claimed to be a good predictor of Birth Asphyxia [8-11]. In the present study, we evaluated the cord blood NRBC Count as a predictor of Birth Asphyxia in term neonates.

In our study, the mean NRBC count was significantly higher in case group as compared to controls, which supports the previously suggested hypothesis that NRBCs are released in the blood in stressful condition of birth asphyxia due to hypoxia. It was also noted that the NRBC count was more in cases who delivered by LSCS and or having meconium stained amniotic fluid. The statistical 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. This suggests that NRBC count increases as the duration and or intensity of hypoxia increases. The similar observations were also noted in previous studies [8-14].

In our study, we analysed the correlation coefficient for NRBC count with APGAR score and cord blood pH. 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 [12]. This shows that as the severity of Birth Asphyxia increases the APGAR score and pH deceases but NRBC count increases.

In our study the NRBC count was found to be a sensitive and specific indicator to predict the birth asphyxia. Similar observation also noted by Colaco et al [12] and Mohanty et al [14].

V. Conclusion

In our study we concluded that the NRBC count in cord blood at birth can be considered as a marker of Birth asphyxia. The number of NRBCs in the cord blood is significantly correlated with the degree of Birth Asphyxia and HIE. This is a simple, cheap, rapid and non-invasive test from the cord blood, which provides valuable information about the well being of the newborn baby at birth and it correlates

well with APGAR score and Cord Blood pH to predict the Birth Asphyxia. The NRBC count can be used as a simple tool to predict the severity of Birth Asphyxia in full term newborns in conditions where APGAR score and or ABG machine with expertise not available and plan the interventions accordingly.

Abbreviations
NRBC: Nucleated Red Blood Cell Count
NNPD: National Neonatal and Perinatal Database
HIE: Hypoxic Ischemic Encephalopathy
SNCU: Special Newborn Care Unit
PIH: Pregnancy Induced Hypertension
LSCS: Lower Segment Caesarean Section
NVD: Normal Vaginal Delivery

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