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Nucleated Red Blood cells as a Marker of Perinatal Hypoxic Events in Neonates

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Abstract

Objectives: To correlate nucleated RBC count per 100 WBC (nRBC/100WBC) within first 24 hours of birth in term and near term neonates with perinatal hypoxic events and to correlate increased nRBC/100WBC with adverse prognostic outcome in neonates. *Design:* Prospective comparative study. *Setting:* Level III neonatal intensive care unit (NICU) of tertiary care hospital in southern India. *Participants:* 128 neonates e— 34 weeks of gestation admitted in NICU over 10 months period. *Methods:* Neonates were grouped into two- perinatal hypoxic and controls. Details regarding birth weight, gestational age, maternal age, relevant antenatal history and mode of delivery was obtained. Examination included Apgar score at birth and assessment for need for any respiratory support. Blood gas estimation from umbilical cord blood at birth and peripheral smear examination for nRBC/100WBC within first 24 hours of birth was done. Morbidity in form of duration of NICU stay was evaluated. *Result:* Among 128 babies, 46 had perinatal hypoxia while 82 acted as control. Mean nRBCs/100WBC among the cases was 15.54±63.66 whereas that in control was 3.74±5.21. Among the term babies, mean nRBC/100 WBC was significantly higher in those with perinatal hypoxia (17.28±69.09) as compared to controls (3.15±4.418). Statistically significant association was observed between mean nRBC/100WBC and gestational age at birth, requirement of resuscitation at birth and duration of NICU stay. On constructing receiver operating characteristics curve, among term babies for nRBC/100 WBC of >2, sensitivity and specificity was 66.67% and 64.15% respectively, with a positive and negative predictive value of 57.77% and 72.34% respectively. The specificity increased to 95% when a cut off > 13 nRBC/100 WBC was used. *Conclusion:* Among term babies, mean nRBC/100WBC was significantly higher in those with perinatal hypoxia as compared to controls. Hence raised nRBC/100WBC at Day 1 of life in term babies can be taken as surrogate marker of perinatal hypoxic event provided confounding factors have been eliminated. Higher mean nRBC/100WBC on Day 1 of life can be used as a predictor of morbidity and hence longer duration of NICU stay.

Keywords: Erythroblasts; Hypoxia; Intensive Care Units; Neonatal; Gestational Age.

The term perinatal asphyxia is used to define asphyxia occurring in utero, during the process of labour, at birth or in the immediate postnatal period. Measures that have been correlated with perinatal asphyxia include: intrapartum electronic fetal monitoring, fetal or umbilical cord pH measurement, meconium-stained amniotic fluid, Apgar score, hypoxic ischemic encephalopathy (HIE), and major organ dysfunction. However, no single marker of perinatal asphyxia has shown good predictive efficiency and only a combination of various indices can act as an early indicator of perinatal asphyxia.

Nucleated red blood cells (nRBC), or erythroblasts, are the premature forms of erythrocytes that are commonly found in the newborn's blood. Their presence in peripheral blood is associated with the hypoxemic nature of fetal growth [1]. They are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as precursors to reticulocytes and mature erythrocytes. In healthy term neonates, the number of erythroblasts approximates 500 nRBC/mm^3 making up 0.1% neonatal RBC. The average number of nRBC/100WBC ranges from 3 to 10 in normal full term infants and upto 25 nRBC/100 WBC in preterm infants [2]. Nucleated RBC count is increased in neonates as a result of acute and chronic hypoxic states [3]. This study was planned with an objective of finding an association between nRBC/100 WBC and perinatal hypoxic event in neonates.

Methods

This was a prospective comparative study conducted in Neonatal Intensive Care (NICU) of Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala from February, 2016 to November, 2016. Serial sampling over a period of ten months was done and the babies ≥ 34 weeks of gestational age admitted to NICU were enrolled in the study after a written informed consent from the parents. Neonates with perinatal hypoxic events were taken as cases while the other babies acted as controls. For the purpose of study, perinatal hypoxic event was defined as either (a)non reassuring fetal heart rate/ non-reactive non stress test or (b) abnormal biophysical profile or (c) doppler abnormalities in form of absent/ reversal of end diastolic umbilical arterial flow or (d) meconium stained amniotic fluid or (e) 1 minute Apgar score <7 or (f) cord umbilical artery pH <7.0 and base excess of ≥ 12 mmol/L or (g) need for resuscitation with bag and mask ventilation and oxygen for > 1 min immediately following birth. Neonates with (a) gestational age of below 34 completed weeks or (b) those admitted in NICU after 24 hours of birth or (c) small or large for gestational age babies or (d) outborn referrals or (e) those with Rh incompatibility setting or (f) neonates with evidence of TORCH infection in the mother or (g) those with congenital malformation and/or chromosomal anomalies were excluded from the study.

Relevant antenatal history in the form of maternal age, period of gestation, gestational diabetes or hypertension, premature rupture or prolonged rupture of membrane, fetal heart rate tracing, biophysical profile, non-stress test results were obtained. Babies were managed at birth as per neonatal resuscitation guidelines. Examination included Apgar score at birth, the need for any respiratory support and assessment of weight. Blood gas estimation from umbilical cord blood at birth and peripheral smear examination for nRBC/100WBC was done within first 24 hours of admission to NICU. Neonates with respiratory distress were given adequate respiratory support.

The primary outcome of the study was to correlate nRBC/100WBC within first 24 hours of birth in term and near term neonates with perinatal hypoxic events and secondary outcome was to correlate increased nRBC/100WBC with adverse prognostic outcome in neonates.

The study was approved by Institutional Research and Ethics Committee and informed consent was obtained from the parents of neonates enrolled in the study.

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) Ver. 20 (IBM Corp, NY). Unpaired t test and ANNOVA were used for values with normal distributions whereas Mann Whitney U test and Kruskal Wallis were applied for skewed distributions. Chi square test was used to compute p- values of 2 X 2 contingency tables. A p-value <0.05 was considered as significant. Receiver-operating characteristic (ROC) curves were also constructed allowing the calculation of positive and negative predictive values.

Results

During the study period of ten months, 128 neonates admitted in NICU were enrolled into the study. Out of which 46 had perinatal hypoxia while 82 acted as controls. Both the groups were comparable for gender, mode of delivery, maternal age, birth weight and Apgar score at 5 min (Table 1). However 84.8% of perinatal hypoxic babies had attained term gestation while only 64.6% of controls had attained term gestation (p value 0.015). Gestational age at birth was significantly more in those with perinatal hypoxia as compared to controls (p value 0.000). Apgar score at 1 min was significantly lower in those with perinatal hypoxia as compared to controls (p value 0.017). Mean cord pH was significantly lower in those with perinatal hypoxia (7.26) compared to control group (7.30) (p value 0.010). However no significant difference in base excess and serum bicarbonate was observed between cases and controls. Mean nRBC/100 WBC in those with perinatal

hypoxia was 15.54±63.66 compared to 3.74±5.21 in among control which was not statistically significant (p value 0.053). Among term babies, mean nRBC/100 WBC was significantly more in babies with perinatal hypoxia than control (p value 0.032). However no such difference was observed among the late preterm babies.

Mean nRBCs/100 WBCs was higher in neonates whose mother did not have gestational diabetes or hypertension which was not statistically significant (Table 2). Mean nucleated RBCs was significantly more in those requiring bag and mask ventilation or intubation as compared to those requiring no resuscitation or those requiring only initial steps of resuscitation (p value 0.013). Also babies with higher mean nucleated RBCs had significantly longer duration of NICU stay (p value 0.001).

Among factors used in the definition of perinatal hypoxic events nucleated RBCs/ 100 WBC was significantly more in the newborns born through meconium stained amniotic fluids, those with umbilical cord base excess >12, those with Apgar score of <7 at 1 minute and those who required bag and mask ventilation for >1 min (Table 3). However no significant difference was observed in nucleated RBCs of babies with abnormal heart rate tracing.

On constructing Receiver Operating Characteristic curve for term babies for nRBC/100WBC of>2, sensitivity and specificity was 66.67% and 64.15% respectively, with a positive and negative predictive value of 57.77% and 72.34 % respectively (Figure 1). The specificity increased to 95 % when a cut off >13 nRBC /100 WBC was used (Table 4).

In univariate analysis, gestational age (coefficient: 0.271; p value 0.015) and duration of NICU stay (coefficient: 0.990; p value 0.000) was significantly associated with mean nucleated RBCs (Table 5). However age of mother, birth weight, Apgar score at 1 and 5 min, cord pH, base excess and bicarbonate were not found to be significantly associated with nucleated RBCs.

Table 1: Baseline characteristics of neonates enrolled

Baseline Characteristics	Perinatal Hypoxic Event	Controls	P-value
Gender (M:F)	22:24	39:43	0.977
Gestation (Term: Late preterm)	39:7	53:29	0.015
Mode of delivery (NVD: C-sec)	17:29	34:48	0.828
Maternal age (yrs), mean (SD)	27.28 (3.87)	28.4 (4.90)	0.145
Birth weight (Kgs), mean (SD)	2.95 (0.39)	2.86 (0.41)	0.195
Gestational age (wks), mean (SD)	38.02 (1.36)	36.97 (1.36)	0.000
Apgar score at 1 min, mean (SD)	8.0 (1.42)	8.59 (0.70)	0.017
Apgar score at 5 min, mean (SD)	8.8 (0.45)	8.9 (0.28)	0.129
Cord pH, mean (SD)	7.26 (0.80)	7.30 (0.07)	0.010
Base excess (mmol/L), mean (SD)	-6.56 (3.47)	-5.47 (2.68)	0.100
$HCO3^-$ (mmol/L), mean (SD)	18.31 (2.47)	19.02 (2.35)	0.115
nRBCs/ 100 WBCs, mean (SD)			
Term neonates	17.28 (69.09)	3.15 (4.41)	0.032
Late preterm neonates	4.83 (6.37)	5.86 (5.98)	0.611
Total	15.54 (63.66)	3.74 (5.21)	0.053

Table 2: Association of population characteristics with nrbc/100wbc

Population Characteristics	N RBC/100WBC, mean (SD)	P- Value
Gestational diabetes		
Present	5.36 (9.70)	0.788
Absent	9.95 (50.40)	
Gestational hypertension		
Present	4.84 (4.26)	0.129
Absent	8.46 (41.36)	
Need for resuscitation		
No resuscitation (n= 113)	7.28 (40.62)	0.013
Initial steps (n= 4)	6.50 (6.65)	
Bag and mask for ≤1 min (n=9)	15.67 (19.35)	
Intubated/BMV >1 min (n=2)	16.00 (5.65)	
Duration of NICU stay		
0-2 days (n= 102)	3.35 (4.33)	0.001
>2-5 days (n=14)	12.14 (15.85)	
>5 days (n=12)	42.50 (123.14)	

Table 3: Association of markers of perinatal hypoxia with nrbc/100wbc

Perinatal Hypoxia Markers	*nRBC/100WBC, mean (SD)*	*P- Value*
Abnormal fetal heart rate tracing		
Present	10.67 (10.06)	0.378
Absent	7.92 (38.98)	
MSAF		
Present	17.39 (71.97)	0.042
Absent	4.34 (5.70)	
Base excess >12		
Present	16.50 (6.36)	0.018
Absent	7.85 (38.83)	
Apgar Score at 1 min <7		
Present	17.63 (18.36)	0.001
Absent	7.34 (39.48)	
Resuscitation (bag and mask for >1min)		
Present	20.00 (18.46)	0.000
Absent	7.29 (39.32)	

Table 4: Sensitivity and specificity and of various nrbc/100wbc cutoffs

Cut-offs	Sensitivity (%)	Specificity (%)
>2	66.67	64.15
>4.35	40.90	80.00
>7.233333333	22.48	90.00
>12.7	12.82	95.00
>15.35	12.82	97.50
>21.94	5.13	99.00

Table 5: Regression coefficients (univariate-linear) between nrbc/ 100wbc and various parameters

Factors	Beta coefficient	p- Value
Gestation (wks)	0.271	0.015
Age of mother (yrs)	0.064	0.462
Birth weight	-0.016	0.877
Apgar at 1 min	0.026	0.841
Apgar at 5 min	0.146	0.338
Duration of NICU stay	0.990	0.000
Cord pH	0.007	0.936
Base excess	0.236	0.179
Bicarbonate	-0.276	0.103

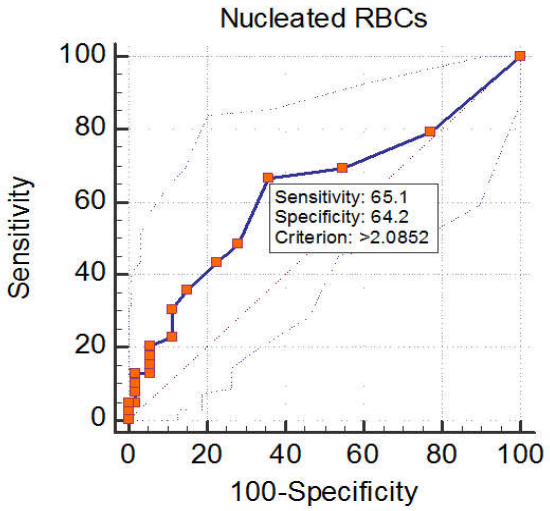

Fig. 1: Roc curve to discriminate the sensitivity and specificity of nrbc/100wbc in the diagnosis of perinatal asphyxia in term babies

Discussion

The study results demonstrate that perinatal hypoxic event was significantly more in term babies as compared to late preterm babies which was similar to the findings by Shireen et al [4]. Probable reason for this finding is using meconium stained amniotic fluid as a surrogate marker for perinatal hypoxia which is more common in term babies than late preterms. Also mean cord pH was lower in babies with perinatal hypoxia than control which was similar to the findings of Boskabadi et al [5].

The mean nRBC/100WBC among late preterm controls in this study was higher than the term controls which was in accordance with the findings of Perrone et al [6]. Mean nRBC/100WBC was significantly increased in term newborns who had a

perinatal hypoxic event compared to controls which was similar to the findings of previous studies [5,7]. However most of the studies have commented on presence of nucleated RBCs in cord blood as a marker of perinatal asphyxia while only few studies have correlated nucleated RBCs in peripheral blood of newborns as a marker of perinatal hypoxia which was done in our study [8]. Mean nRBC/100 WBC was not significantly different between late preterm cases and controls which was contrary to the findings of Krishna P V [9].

The study demonstrated that mean nRBCs/100 WBCs was lower in the newborns whose mother had gestational diabetes which was contrary to the findings of Yeruchimovich et al [10]. This was possibly due to the fact that babies born to mothers with uncontrolled gestational diabetes leading to large or small for gestational age babies were excluded from the study. Also because the babies born to mothers with gestational diabetes were distributed as both cases and controls but primarily acted as controls. Also this study demonstrated that mean nRBC/100 WBC was lower in babies born to mothers with gestational hypertension which was contrary to the findings of Hebbar et al [11]

This study revealed mean nRBC/100WBC was significantly more in those requiring bag and mask ventilation or intubation as compared to those requiring no resuscitation or those requiring only initial steps of resuscitation (p value 0.013). This finding was in accordance with the fact that perinatal hypoxic even was defined as bag and mask ventilation > 1 min. Also babies with higher mean nucleated RBCs had significantly longer duration of NICU stay (p value 0.001) which was in accordance with the study by Gasparovic et al [12].

In our study no significant association was found between nucleated RBC and abnormal fetal heart rate tracing which was contrary to the findings of Ferber et al [13]. Nucleated RBCs/100 WBC was significantly correlated with meconium stained amniotic fluid at birth which was in accordance with previous studies [14]. Also Apgar score of <7 at 1 min was significantly correlated with increased nucleated RBC/ 100 WBC which similar to the findings of Hanlon- Lundberg et al [15].

In this study among term babies using a nRBC/ 100 WBC of >2, sensitivity and specificity was 66.67% and 64.15% respectively, with a positive and negative predictive value of 57.77% and 72.34 % respectively. The specificity increased to 95% when a cut off > 13 nRBC /100 WBC was used. This study therefore gave a cut off of 13 nRBC/100WBC can be used as an additional marker to suggest perinatal hypoxia in term newborns when in diagnostic dilemma.

The study was limited by the fact that only newborns admitted in NICU were included in the study and normal healthy newborns not requiring NICU admission were not taken thereby giving a biased control population.

In conclusion raised nRBC/100WBC in peripheral blood at Day 1 of life in term babies can be taken as surrogate marker of perinatal hypoxia provided confounding factors have been eliminated. Higher mean nucleated RBC count on Day 1 of life can be used as a predictor of morbidity and correlated with consequently longer duration of NICU stay.

What this study adds?

- Nucleated RBC count per 100 WBC of >13 has 95% specificity for predicting perinatal hypoxic event in neonates.

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Contributors

DT: Carrying out the research work and preparing the manuscript; JA: Supervising the research work and proof reading manuscript; JJ: Guiding in preparing the protocol and manuscript of research work.

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