

Nucleated red blood cell counts in asphyxiated newborns

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To cite this articleTungalag. L, Gerelmaa. Z. Nucleated Red Blood Cell Counts in Asphyxiated Newborns. *Open Science Journal of Clinical Medicine*. Vol. 2, No. 1, 2014, pp. 33-38.**Abstract**

The purpose of this study was to investigate variations in nucleated red blood cell count (NRBC) in blood associated with perinatal asphyxia. A total of 186 infants completed the study (cases n=62 (33.3%), controls n=124 (66.7%). Levels of nucleated red blood cell per 100 white blood cells and absolute nucleated red blood cell counts in umbilical venous blood were compared for asphyxiated and normal neonates. The mean NRBC/100 WBC count for case group 11.36±10.7, control group was 4.83±3.01, absolute NRBS count case group 1.75±2.44, control group was 0.74±0.62 (P<0.000). In present study NRBC/100WBC and absolute NRBC were significantly higher in neonates with birth asphyxia, and high levels of NRBC were associated with a low APGAR score, MSAF, fetal distress, degree of HIE (P<0.000). Hence NRBC count can be a useful part of the obstetrician's armamentarium for the evaluation of perinatal asphyxia where facilities of pH sampling are not available and can serve as are liable, inexpensive and easily available marker of perinatal asphyxia.

Keywords

Asphyxia, Hypoxic Ischemic Encephalopathy, Nucleated Red Blood Cells (NRBC)

1. Introduction

Asphyxia is a major cause of acute mortality and chronic neurologic disability amongst survivors, and is a complication that occurs between 2 – 10% of deliveries.¹ Each year approximately 4 million babies are born asphyxiated. Of these, an estimated 1.2 million die and least same number develop severe consequences such as cerebral palsy, epilepsy and developmental delay.^{2,3} Hypoxic ischemic encephalopathy (HIE) is the most severe result of perinatal asphyxia. Of patients with HIE, 15-20% die in the neonatal period, and 25-30% of the survivors develop permanent neurological disorders, such as cerebral palsy (CP) and mental retardation.⁴ Parameters that have been used to predict or define perinatal asphyxia include: intra-partum electronic fetal monitoring, fetal or umbilical cord pH measurement, meconium stained amniotic fluid (MSAF), APGAR score, hypoxic ischemic encephalopathy

(HIE), and major organ disorder. NRBC count in umbilical venous blood of neonates has been reported as a possible marker of perinatal asphyxia.⁵⁻¹⁵ Nucleated red blood cells (NRBC) are commonly seen in the blood of neonates. 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. Because 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.

2. Materials and Methods

A prospective, case control study was conducted between October 2012 and September 2013, in Maternity Hospital 3, Ulaanbaatar. The study was approved by the Human Research and Ethics Committee of Health Sciences

University of Mongolia. All 4376 infant's was born during the duration of the study, among 186 neonates who were originally recruited into the study.

2.1. Inclusion Criteria

Perinatal asphyxia was defined as the presence of at least 2 of the following conditions:

1. Signs of fetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability)
2. Thick, MSAF and respiratory depression, hypotonia, or bradycardia
3. APGAR score of 4 or less at one minute or 7 or less at five minutes
4. A need for resuscitation for more than 1 minute with positive pressure ventilation and oxygen immediately after birth.

The entry criteria for these normal non-asphyxiated newborns were as follows: appropriate for gestational age neonate at more than 37 weeks gestation, birth weight >2500 g, APGAR score >7 at both one and five minutes, normal intra-partum fetal heart rate (FHR) pattern, clear amniotic fluid.

2.2. Clinical Assessment

The neurologic examination used to evaluate neurologic function of the term neonates with asphyxia at birth, 2 days of life. According to the criteria of SARNAT, HIE was classified:

1. As mild (Grade 1) if hyper-excitability, hyper-alertness, or hyperreflexia persisted without seizures for at least 24 hours after birth;
2. As moderate (Grade 2) if the infant was lethargic, had hypotonia, weak primitive reflexes, pupil miosis, and seizures;

3. As severe (Grade 3) if the infant had apnea, flaccid weakness, frequent seizures, decelerated posture, or coma. The outcome was classified as favorable or adverse. A favorable outcome was defined as normal neurological development and good general condition at the end of the first month.

2.3. Laboratory Measurement

For complete blood cell count (CBC) and NRBC counts, 2 ml of umbilical blood were collected and delivered in sterile tubes that contained K3-EDTA anticoagulant. CBCs and NRBCs were measured using SYSMEX – 21 calibrated electronic counter. Data processing was done by using statistical program SPSS-17.

3. Results

Among 186 neonates who were originally recruited into the study. The median birth weight of the children was 3381 g and median gestation age was 38.95 weeks. There was no statistically significant difference ($P>0.05$) between the two groups regarding gender, maternal age, and maternal parity. However, case group significantly greater ($P=0.002$) gestational age and birth weight (Table 1).

The mean Apgar score 1 minutes for case group 4.93 ± 1.51 , control group was 6.99 ± 4.93 ($P<0.000$), the mean APGAR score 5 minutes for case group 6.56 ± 1.17 , control group was 8.38 ± 0.5 ($P<0.000$). Laboratory findings of same blood count parameters revealed differences between asphyxiated and healthy newborns, also at gender. The mean NRBC/100 WBC count for case group 11.36 ± 10.7 , control group was 4.83 ± 3.01 ; absolute NRBS count case group 1.75 ± 2.44 , control group was 0.74 ± 0.62 (Table 2).

Table 1. Clinical characteristics of the studied population

	Control group N=124	Study group N=62	P-value
Age of mother	26.93±5.69	28.98±6.72	0.062
Birth weight	3468.55±435.4	3193.30±575.4	0.002
Gander			
• Male	60±1.00	34±1.00	0.149
• Female	64±2.00	28±2.00	0.148
Gestation age	39.2±1.07	38.3±2.11	0.002
Lying-in:			
• Primipara	48	19	
• Multipara	76	43	
Mode of delivery :			
• SVD	82	31	
• EMCS	20	22	
• ELCS	22	9	
APGAR score 1 min	6.99±0.27	4.93±1.51	0.000
APGAR score 5 min	8.38±0.52	6.56±1.17	0.000

Table 2. Comparison of NRBC count values

Group Statistics				
	asphyxia	N	Mean±SD	P-value
NRBC/100WBC	no	124	4.836±3.0156	0.000
	yes	62	11.354±10.2382	
Absolute NRBC	no	124	0.7467±0.62002	0.000
	yes	62	1.6528±2.36189	

Table 3. Correlation between NRBC counts and APGAR 1 minute in cases

Correlations					
		Apgarscore1	Apgarscore5	NRBC#	NRBC%
Apgarscore1	Pearson Correlation	1	.881**	-.302**	-.084
	Sig. (2-tailed)		.000	.000	.326
Apgarscore5	Pearson Correlation	.881**	1	-.282**	-.144
	Sig. (2-tailed)	.000		.001	.092
NRBC#	Pearson Correlation	-.302**	-.282**	1	.474**
	Sig. (2-tailed)	.000	.001		.000
NRBC%	Pearson Correlation	-.084	-.144	.474**	1
	Sig. (2-tailed)	.326	.092	.000	

** . Correlation is significant at the 0.01 level (2-tailed).

Table 4. NRBC counts in the elective caesarean section, spontaneous vaginal delivery and emergency caesarean section groups

Group Statistics				
	Mode of delivery	N	Mean±SD	P-value
NRBC/100WBC	delivery	93	6.69±7.57	0.62
	elective CS	17	7.45±7.12	
	emergency CS	25	7.37±5.68	
Absolute NRBC	delivery	93	1.09±1.70	0.89
	elective CS	17	0.83±0.82	
	emergency CS	25	1.13±1.21	

Table 5. NRBC count in the fetal distress group and normal group

Group Statistics				
	Fetal distress	N	Mean±SD	P-value
NRBC/100WBC	no	135	5.587±4.0477	0.001
	yes	51	9.547±10.0387	
Absolute NRBC	no	135	0.8191±0.69790	0.016
	yes	51	1.4569±2.28746	

Table 6. Comparison of NRBC count between neonate MSAF and clear amniotic fluid

	Group Statistics			P-value
	MSAF	N	Mean±SD	
NRBC/100 WBC	no	138	5.962±7.5530	0.013
	yes	48	9.083±5.7063	
Absolute NRBC	no	138	0.8276±0.95125	0.015
	yes	48	1.4806±2.17476	

Table 7. NRBC count and degree HIE

	HIE(-)	HIE1	HIE2	HIE3
N	14	12	11	8
NRBC mean	2.56	9.1	14.64	23.01
NRBC (SD)	1.13	3.69	2.14	18.21
NRBC range	2.8	14.7	8.1	56.5

Table 8. Area Under the Curve

Area	P-γтга	NRBC#	
		Lower	Upper
.754	.000	.664	.843

Null hypothesis: true area = 0.5

This difference was highly significant. It was observed that a very strong and negative correlation exists between APGAR at 1, 5 minutes and NRBC/100WBC count ($r = -2.84$, $P < 0.000$, Table 3).

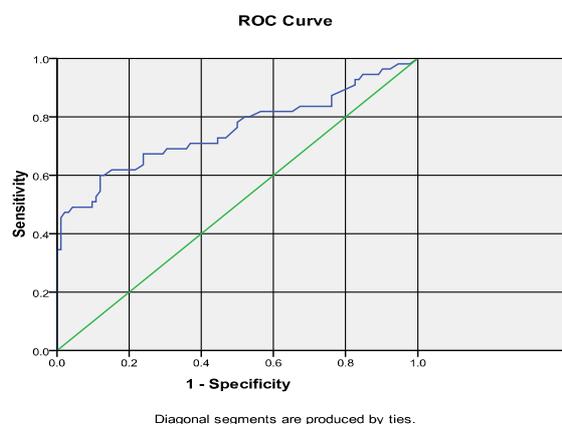
The finding of this prospective study that labor is associated with umbilical cord blood NRBC counts. There was no significant association between the duration of labor and NRBC counts (Table 4).

The mean NRBC/100WBC count in the fetal distress group was 9.547 ± 10.0387 , and normal group was 5.587 ± 4.0477 , mean absolute NRBC count in the fetal distress group was 1.456 ± 0.697 , and normal group was 0.819 ± 0.697 . This difference was highly significant ($P < 0.001$, Table 5).

Of this study we sought to determine the correlation between NRBC counts and MSAF to establish NRBC determination as an important indicator of asphyxia.

Among 45 infants with perinatal asphyxia, 14 infants had asphyxia without HIE, 12 had an HIE grade 1, 11 had grade 2, and 8 had grade 3. NRBC counts were found to be 4.83 ± 3.01 for healthy infants, 2.56 ± 1.13 for asphyxiated neonates without HIE, 9.1 ± 3.69 for HIE grade 1, 14.64 ± 2.14 for HIE grade 2, 23.01 ± 18.21 for HIE grade 3. There was a significant relationship between NRBC count and the degree of encephalopathy.

The significance of determining the above mentioned NRBC level in assessment and prediction of perinatal asphyxia could be seen from the results of ROC curve test (significance and sensitive area = 0.75, $p < 0.000$, Figure 1, Table 8)

**Figure 1.** Sensitivity and specificity of NRBC count in the diagnosis of perinatal asphyxia

According to the Binary Logistic Regression test the risk factors that associated with asphyxia were: early toxicity ($OR = 3.926$, $p < 0.061$), preeclampsia ($OR = 5.390$, $p < 0.001$), oligohydramnios ($OR = 11.286$, $p < 0.083$), FHR decrease during delivery ($OR = 47.964$, $p < 0.000$), FHR increase during delivery ($OR = 8.464$, $p < 0.069$).

4. Discussion

Although NRBCs rarely circulate in older children, they are commonly seen in the blood of neonates. In normal newborns less than 10 NRBC/100WBC is within normal limit.⁶ Hypoxia can be acute or chronic, however the

mechanism leading to raised NRBC count. In our study the mean NRBCs in asphyxiated neonates was 11.36 ± 10.7 when compared to 4.83 ± 3.01 in non-asphyxiated neonates. Several authors have evaluated the relationship between various surrogate markers of asphyxia and NRBC count. In the study of Hanlon-Lundenberg et al the NRBC count in term singleton neonates was 8.55 ± 10.27 ,^{17,20} and that in the study of Saracoglu et al was 7.56 ± 3.85 .^{15,16} Phelan et al found the mean NRBCs of 3.4 ± 3.0 per 100 WBCs in non-asphyxiated neonates and 34.5 ± 68.3 in asphyxiated neonates.^{6,18} Spencer et al concluded that NRBC count is better marker of fetal metabolic acidosis than MSAF,²¹ non-reassuring FHR,^{22,23} low APGAR scores and fetal erythropoietin levels. The mean NRBC count was higher in MSAF than in clear liquor and was statistically in our study. Similar observation was made by Doll-berg et al²⁶ found increased NRBC count in neonates with meconium aspiration syndrome.

In present study RBC/100WBC and absolute NRBC were significantly higher in neonates with birth asphyxia, and high levels of NRBC were associated with a low APGAR score, MSAF, fetal distress, degree of HIE. Our study was 45 infants with perinatal asphyxia, 14 infants had asphyxia without HIE, 12 had an HIE grade 1, 11 had grade 2, and 8 had grade 3. NRBC counts were found to be 4.83 ± 3.01 for healthy infants, 2.56 ± 1.13 for asphyxiated neonates without HIE, 9.1 ± 3.69 for HIE grade 1, 14.64 ± 2.14 for HIE grade 2, 23.01 ± 18.21 for HIE grade 3. There was a significant relationship between NRBC count and the degree of encephalopathy. Saracoglu et al. also evaluated this relationship and found a significant correlation ($P < 0.001$).^{15,16}

Hanlon-Lundberg and Kirby evaluated the relation between the severity of asphyxia and increased NRBCs by comparing cord NRBCs with cord pH and APGAR scores. The NRBC counts increased with progressive increases in cord acidosis and with progressive decreases in the APGAR scores.¹⁷

5. Conclusions

- Nucleated red blood cell can be used as surrogate prediction for perinatal asphyxia.
- Increase nucleated red blood cells are seen in asphyxiated newborns.
- There is significant difference between the NRBCs among cases and control groups.
- Estimation of NRBCs in the cord blood can help distinguish an asphyxiated from a non-asphyxiated term neonate and predict occurrence of HIE.
- This simple test maybe helpful the rapid assessment of asphyxia.
- In many settings, especially resource-poor countries, it may be impossible to assess fetal or neonatal acidemia. Hence NRBC count can be a useful part of the obstetrician's armamentarium for the evaluation of perinatal asphyxia where facilities of

pH sampling are not available and can serve as a reliable, inexpensive and easily available marker of perinatal asphyxia.

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