

Nucleated Red Blood Cell in Cord Blood as a Marker of Perinatal Asphyxia

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ABSTRACT

Background: Perinatal asphyxia is a major cause of neurological morbidity and mortality in India. The purpose of this study was to investigate variations in nucleated red blood cell (NRBC) in blood associated with perinatal asphyxia and its relationship to both the severity and short term prognosis of asphyxia. **Methods:** A prospective (case-control) study was undertaken at Gandhi Medical College and Associated Hospitals. A total of 100 neonates were included in the study. Levels of NRBC/100 white blood cells (WBC) and absolute NRBC counts in cord blood were compared for 50 asphyxiated (case group) and 50 normal neonates (control group). These parameters were also related to the severity of asphyxia and clinical outcome. **Results:** The number of NRBC/100 WBC in the blood of 50 newborns each in the asphyxiated and in the control group were mean 29.5 ± 26.0 , range 7-144 NRBCs/100 WBC and mean \pm standard deviation 5.9 ± 2.6 , range 3-14 NRBCs/100 WBC respectively ($P < 0.01$). Using quartile deviation, staging of hypoxic ischemic encephalopathy (HIE) was done on basis of NRBC count and there was 80% agreement between clinical and NRBC staging of HIE. There was a significant ($P < 0.01$) correlation of the number of NRBC\100 WBC with Apgar scoring, HIE staging and mortality. **Conclusions:** The NRBCs/100 WBCs can be used as a simple marker for the assessment of severity and early outcome of perinatal asphyxia.

Key words:

Asphyxia, hypoxic ischemic encephalopathy, nucleated red blood cells

INTRODUCTION

Perinatal asphyxia is an insult to the fetus or newborn due to lack of oxygen (hypoxia) and/or lack of perfusion to various organs. Diagnosis of hypoxic ischemic encephalopathy (HIE) requires an abnormal neurological examination on the 1st day of birth and evidence of an asphyxiating event taking place in the perinatal period.^[1] In asphyxiated neonates there are many biochemical and hematological variations like acidosis, abnormal electroencephalogram, altered blood flow, hypoxia and hypercarbia etc., Many studies in recent past have suggested that an increase number of nucleated red blood cells (NRBC) in umbilical cord blood may be a useful marker to identify birth asphyxia^[2] as shown in Figure 1.

In our study, we have made an attempt to know the importance of this hematological variation. The value of NRBCs count estimation as the method of intrapartum fetal monitoring, by the use of fetal scalp blood sampling needs to be evaluated.

METHODS

This prospective case-control study was performed on 100 newborns, out of which 50 were cases and rest 50 were taken as control. The cases were selected on the basis of evidence of fetal distress as assessed by fetal heart

rate monitoring or history of meconium stained liquor and who required resuscitation at birth and with 5 min Apgar^[3] score ≤ 3 . Premature newborns and newborns presenting by breach or born to mothers with diabetes or with Rh isoimmunization were excluded. In 50 consecutive asphyxiated neonates, mixed cord blood samples were taken from the placental side of the cut cord by milking method into an ethylenediaminetetraacetic acid containing tube along with a drop of blood put on a glass slide and smear made. The smear was immediately fixed with methylalcohol.

After the resuscitation was complete and baby stabilized, the baby was shifted to neonatal intensive care unit (NICU) for further management. Once admitted, these babies were

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Access this article online

Quick Response Code:



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www.jcnonweb.com

DOI:

10.4103/2249-4847.123097

managed as per NICU protocols. During the hospital stay, the babies were followed-up and different clinical events during the stay were recorded and the outcome noted. The staging of HIE was done according to Sarnat and Sarnat.^[4]

The blood samples were analyzed for hemoglobin%, total leukocyte count, differential leukocyte count, hematocrit and erythrocyte sedimentation rate. The smears were stained with Leishman stain and number of NRBCs/100 white blood cells (WBC) was determined. These reports were observed and then analyzed for immediate correlation with different stages of HIE. During the same period, cord blood samples of normal newborn which acted as controls were taken and subjected to similar hematological evaluation. These babies were also followed for 7 days or upto discharge and evaluated clinically.

Statistical analysis

Chi-square analysis of variance (for qualitative analysis), Student’s *t*-test (for comparing mean NRBC in different stages of HIE), Mann-Whitney test and Quartile deviation (for HIE staging based on NRBC) and Levene’s test for equality of variances.

OBSERVATIONS AND RESULTS

88% of our cases were in either stage II or stage III and most of these case had Apgar score of 3 or < 3. The asphyxiated group had a significantly higher number of NRBCs (mean 29.5 ± 26.0 and range 7-144 NRBCs/100 WBC) when compared to the non-asphyxiated group (mean ± SD 5.9 ± 2.6 and range 3-14 NRBCs/100 WBC) and this observation had *P* < 0.01 and *t* = 6.38 as depicted in Table 1.

After the above observation, 10 was taken as a cut-off value between normal and asphyxiated group, and they were grouped as >10 and <10. It was noticed that 47 cases were true positive

and one case was false positive with respect to value > 10. With cut-off value < 10, three cases were false negative and 49 cases were true negative, indicating no difference in both categories. *P* < 0.01, Chi-square value = 84.76, degree of freedom = 1 was observed which was statistically significant with odd ratio 768 (95% confidence interval 78-7555) as shown in Table 2. When degree of validity of NRBCs was further analyzed, it had a sensitivity of 94%, specificity of 98%, positive predictive value of 98%, negative predictive value of 94% with an overall accuracy of 96%. Higher the stage of HIE greater was the nucleated RBC count as shown in Table 3.

Using quartile deviation, staging of HIE was done on basis of NRBC count in which stage I HIE it was <16 NRBC/100 WBC, stage II HIE it was 16-27 NRBC/100WBC and stage 3 HIE >27 NRBC/100 WBC. On using the quartile deviation, 9 cases (18%) fell in stage I HIE, 24 cases (48%) in stage II HIE and 17 cases (34%) in stage III HIE in comparison to clinical staging by Sarnat and Sarnat staging in which

Table 1: Nucleated RBCs/100 WBCs in asphyxiated and non-asphyxiated neonates

Nucleated RBC/100 WBC	Asphyxiated group (%)	Non-asphyxiated group (%)
0-10	3 (6)	49 (98)
11-50	41 (82)	1 (2)
51-100	4 (8)	Nil
101-150	2 (4)	Nil
Total	50	50
Mean ± SD NRBC	29.5 ± 26.0	5.9 ± 2.6
Range	7-144 NRBC/100 WBC	3-14 NRBC/100 WBC
Statistical significance	<i>t</i> = 6.38 <i>P</i> < 0.01	

RBC – Red blood cell; WBC – White blood cell; NRBC – Nucleated red blood cell; SD – Standard deviation

Table 2: Diagnostic validity of nucleated RBCs in HIE

Nucleated RBC/100 WBC	Asphyxia		Total
	Present	Absent	
> 10	47 ^{TP}	1 ^{FP}	48
< 10	3 ^{FN}	49 ^{TN}	52
Total	50	50	100

RBC – Red blood cell; WBC – White blood cell; HIE – Hypoxic ischemic encephalopathy; TP – True positive; FN – False negative; FP – False positive; TN – True negative

Table 3: Nucleated RBC in different stages of HIE

HIE stages	Nucleated RBC/100 WBC	
	Range	Mean ± SD
I	7-13	10.17 ± 2.64
II	13-48	19.04 ± 6.86
III	17-144	45.14 ± 32.41
Total	7-144	29.5 ± 26.0

RBC – Red blood cell; WBC – White blood cell; HIE – Hypoxic ischemic encephalopathy; SD – Standard deviation

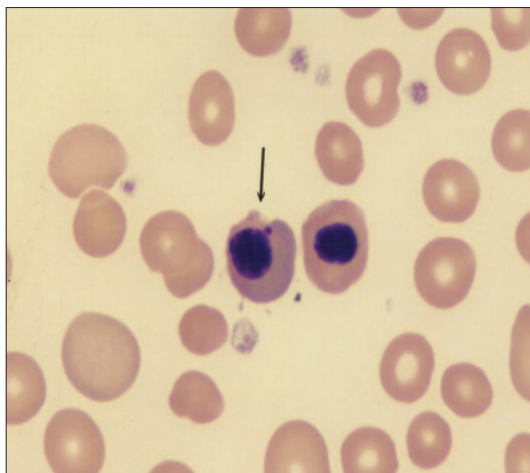


Figure 1: Nucleated red blood cells as seen in the peripheral smear of cord blood

6 cases (12%) fell in stage I, 22 cases (44%) each in stage II and stage III. There is an 80% agreement between clinical and NRBC staging of HIE (by Quartile) as observed in Table 4. At Apgar of 2, mean NRBC/100 WBC was 80.8 ± 52.1 , at Apgar of 3 mean NRBC count/100 was 28 ± 13.2 . These values indicate that lower the Apgar score, higher was mean NRBC/100 WBC. In this relationship, $t = 2.258$ and $P = 0.0142$ which is statistically significant. When the mean nucleated RBC count was compared between the neonates who survived and who expired after sustaining asphyxia, it was seen that the variation was statistically significant ($P = 0.001$). The mean NRBC count in the “expired” group was higher (43.19 ± 33.59) as compared to the “alive” group (19.51 ± 11.51) as shown in Tables 5a and b.

The NRBC count was compared between SFD babies and AFD babies of our study population. It was seen that mean nucleated RBC in SFD babies was significantly higher (47.73 ± 30.6) than that of AFD babies (21.63 ± 24.25) in the asphyxiated group $t = 2.932$, $P = 0.0025$. However, no such statistically significant variation was observed in the non-asphyxiated group between SFD and AFD babies $P = 0.256$ as shown in Table 6.

DISCUSSION

NRBCs are a common observation in the circulating blood of newborn. 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. Many acute and chronic stimuli cause increase in the number of circulating NRBCs from either increased erythropoietic activity or a sudden release from the marrow storage pools.^[5] The number of NRBC/100 WBC is quite variable, but is rarely more than 10.^[6-8] There are instances in which the number of NRBCs exceeds 10, the most frequent causes are prematurity, Rhesus sensitization and maternal diabetes mellitus. In our study, all these conditions have been excluded as exclusion criteria. Perinatal asphyxia has also been suggested to induce a rise in the number of nucleated RBC in the cord blood of newborn.

In the present study, the non-asphyxiated neonates i.e., control group had a mean \pm SD of 5.9 ± 2.6 NRBC range of 3-14 NRBC for 100/WBC, the asphyxiated group had a mean \pm SD of 29.5 ± 26.0 NRBC and a range of 7-144 NRBC for 100/WBC, and it had a $P < 0.001$. Similar observations were made by Phelan *et al.*^[6] and Korst *et al.*^[9] Spencer *et al.*,^[10] in their study of 14 and 78 asphyxiated and non-asphyxiated neonates respectively, found that asphyxiated group had a high NRBC count than the control group. Buonocore *et al.*^[11] in his study concluded that increase in NRBC count at birth not only reflects a response of the infant to perinatal hypoxia but is also a reliable index of perinatal brain damage.

Table 4: Staging of HIE on basis of nucleated RBC count using quartile deviation and its correlation with clinical staging

Nucleated RBC count/100 WBC	HIE stages by quartile deviation	No. of cases (%) using quartile deviation	Clinical staging of HIE by Sarnat of Sarnat staging (%)		
			I	II	III
<16	I	9 (18)	6	3	-
16-27	II	24 (48)	-	18	6
>27	III	17 (34)		1	16
Total		50	6 (12)	22 (44)	22 (44)

NRBC – Red blood cell; WBC – White blood cell; HIE – Hypoxic ischemic encephalopathy

Table 5a: Mortality in asphyxiated group in relation with nucleated RBCs

Outcome	Number	Mean (NRBC)	Standard deviation	Standard error mean
Alive	29	19.51	11.51	2.13
Expired	21	43.19	33.59	7.33

NRBC – Nucleated red blood cell; RBC – Red blood cell

Table 5b: Mortality in asphyxiated group in relation with nucleated RBCs

NRBC	Independent samples test				
	Levene's test for equality of variance		T test for equality of means		
	F	P value	T	df	97% of confidence interval of difference
					Lower Upper
Equal variances assumed	12.487	0.001	-3.53	48.0	-37.15 -10.19
Equal variances not assumed			-3.100	23.4	-39.45 -7.89

NRBC – Nucleated red blood cell; RBCs – Red blood cells

Table 6: NRBC count between SFD babies and AFD babies

Groups	AFD babies		SFD babies	
	Number	Mean \pm SD	Number	Mean \pm SD
Asphyxiated	35	21.63 \pm 24.25	15	47.73 \pm 30.6
Non-asphyxiated	30	4.83 \pm 2.8	20	7.45 \pm 2.35

NRBC – Nucleated red blood cell; AFD – Appropriate for date; SFD – Small for date

We evaluated the relationship between HIE staging and nucleated RBC in our study and found that higher the HIE staging, higher was the mean NRBC/100 WBC. It was observed that in HIE stage 1 mean NRBC/100 WBC was 10.17 ± 2.64 . It was 19.04 ± 6.86 and 45.14 ± 32.41 for stages II and III respectively. Similarly lower the Apgar score higher was the number of nucleated RBCs.

It was also observed that there is a statistically significant difference in NRBC count between the surviving asphyxiated neonates and in the neonates who expired with a higher

count in the latter. Increase in NRBC count as marker for fetal asphyxia in our study had a sensitivity of 94%, specificity of 98%, positive predictive value of 98%, negative predictive value of 94% with an overall accuracy of 96%.

CONCLUSION

NRBCs/100 WBCs is a simple laboratory test which is easy to perform, cost-effective and highly reliable. The cord blood NRBCs/100 WBCs has a potential of being used as a simple marker for determining the severity and predicting the in hospital pre-discharge outcome of fetal asphyxia. Further studies are required to determine its value as a part of scoring system.

ACKNOWLEDGMENT

The authors hereby acknowledge the efforts made by Dr. Bhupesh Kumar MD (P.S.M.), Scientist Indian Council of Medical Research for assisting in data analysis.

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How to cite this article: Goel M, Dwivedi R, Gohiya P, Hegde D. Nucleated red blood cell in cord blood as a marker of perinatal asphyxia. J Clin Neonatol 2013;2:179-82.

Source of Support: Nil, **Conflict of Interest:** None declared.

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