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 ± 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[1] 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[1] 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
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.

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/100 WBC 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 RBCs/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    | \( t = 6.38 \)             | \( P < 0.01 \)              |

| Table 2: Diagnostic validity of nucleated RBCs in HIE |
|-----------------------------|-----------------------------|-----------------------------|
| Nucleated RBCs/100 WBC      | Asphyxia                   | Absent                     |
| Present                     | Absent                     | Total                       |
| >10                         | 47FP                       | 1FP                         | 48                          |
| <10                         | 3FN                        | 49FN                        | 52                          |
| Total                       | 50                         | 50                          | 100                         |

| Table 3: Nucleated RBC in different stages of HIE |
|-----------------------------|-----------------------------|
| HIE stages                  | Nucleated RBCs/100 WBC      |
| Range                       | Mean±SD                     |
| I                           | 7-13                        | 10.17±2.64                  |
| II                          | 13-48                       | 19.04±6.86                  |
| III                         | 17-144                      | 47.14±32.41                 |
| Total                       | 7-144                       | 29.5±26.0                   |

*Figure 1: Nucleated red blood cells as seen in the peripheral smear of cord blood*
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.\(^\text{[5]}\) The number of NRBC/100 WBC is quite variable, but is rarely more than 10.\(^\text{[6-8]}\) 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 ± SD of 5.9 ± 2.6 NRBC range of 3-14 NRBC for 100/WBC, the asphyxiated group had a mean ± 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.\(^\text{[6]}\) and Korst et al.\(^\text{[6]}\) 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.\(^\text{[11]}\) 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.

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.

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REFERENCES

1. Synder EY, Cloherty JP. Perinatal asphyxia. Manual of Neonatal Car. 6th ed. Lippincott-Williams and Wilkins; 2008. p. 518-28.
2. Hermansen MC. Nucleated red blood cells in the fetus and newborn. Arch Dis Child Fetal Neonatal Ed 2001;84:F211-5.
3. Apgar V. A proposal for a new method of evaluation of the newborn infant. Curr Res Anesth Analg 1953;32:160-7.
4. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976;33:696-705.
5. Ruth V, Fyhrquist F, Clemens G, Raivio KO. Cord plasma vasopressin, erythropoietin, and hypoxanthine as indices of asphyxia at birth. Pediatr Res 1988;24:490-4.
6. Phelan JP, Ahn MO, Korst LM, Martin GI. Nucleated red blood cells: A marker for fetal asphyxia? Am J Obstet Gynecol 1995;173:1386-4.
7. Green DW, Mimouni F. Nucleated erythrocytes in healthy infants and in infants of diabetic mothers. J Pediatr 1990;116:29-31.
8. McCarthy JM, Capullari T, Thompson Z, Zhu Y, Spellacy WN. Umbilical cord nucleated red blood cell counts: Normal values and the effect of labor. J Perinatol 2006;26:89-92.
9. Korst LM, Phelan JP, Ahn MO, Martin GI. Nucleated red blood cells: An update on the marker for fetal asphyxia. Am J Obstet Gynecol 1996;175:843-6.
10. Spencer MK, Khong TY, Matthews BL, MacLennan AH. Haematopoietic indicators of fetal metabolic acidosis. Aust N Z J Obstet Gynaecol 2000;40:286-9.
11. Buonocore G, Perrone S, Gioia D, Gatti MG, Massafr A, Agosta R, et al. Nucleated red blood cell count at birth as an index of perinatal brain damage. Am J Obstet Gynecol 1999;181:1500-5.

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