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

Kanodia P¹, Bhatta NK², Singh RR³, Shah GS⁴, Yadav SP⁵, Yadav SK⁶

Abstract

Introduction: Perinatal asphyxia is a common problem with the incidence varying from 0.5 –2% of live births. According to World Health Organization, approximately 4 million babies die each year before they reach the age of one month. The number of NRBC/100 WBC is variable but is rarely greater than 10 in normal neonates. This simple test can be helpful in the rapid assessment of perinatal asphyxia. Material and Methods: This prospective case-control study and there were 82 newborns in Case and 82 newborns in Controls comprising of asphyxiated and non-asphyxiated neonates, respectively, over a period of 12 months. Results: Out of the 82 neonates in case group, fifty nine (59) neonates were found to have NRBC level ≥10/100 WBC, out of which 58 (70.7%) were cases and 1 (1.2%) was a control. NRBC count of ≥10/100 WBC were seen more in the newborn who had low 5 min Apgar score and in the newborn with severe HIE, these association were statistically significant (P value <0.001). The cut-off NRBC value of ≥10/100 WBC also found to have a sensitivity of 70.30% with a specificity of 98.78%. NRBC has a positive predictive value of 98.31% with a negative predictive value of 77.14%. Significance and sensitive area for ROC curve was 0.875. The ROC curve was calculated with cut-off NRBC value of ≥10/100 WBC. Conclusions: NRBC counts can be very useful to differentiate HIE newborns from non-HIE newborns which will help in appropriate management and better outcome of these newborns.

Key words: Apgar score, HIE, NRBC count, Perinatal Asphyxia

Introduction

Perinatal asphyxia is a common problem with the incidence varying from 0.5 –2% of live births\(^1\),\(^2\),\(^3\),\(^4\). The highest annual neonatal deaths are in South Asia, where an estimated 51 deaths occur for every 1,000 live births\(^1\) amongst which 21.0% of newborn mortality occurs due to birth asphyxia\(^5\). Failure to initiate and sustain breathing immediately after delivery has been associated with hypoxic ischemic injury to the central nervous system (CNS) and the clinical manifestations of this injury have been termed as Hypoxic Ischemic Encephalopathy (HIE). Nucleated Red Blood Cell (NRBC) counts in umbilical blood of neonates have been reported as a possible marker of perinatal asphyxia\(^6\). The number of NRBC per 100 WBC is variable but is rarely greater than 10 in normal neonates\(^5\),\(^7\),\(^8\),\(^9\),\(^10\),\(^11\). 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.

The aim of the study was to find out the association of nucleated RBC with perinatal asphyxia, HIE and Apgar score...
Material and Methods

This prospective case-control study was performed on 164 term newborns, out of which 82 were cases and rest 82 were control. This study was conducted at Neonatology division of BP KIHS. Study period was for one year from May 2014 to May 2015. The cases were selected as per WHO definition- “failure to initiate and sustain breathing at birth,” Apgar score of 6 or less at 5 minute, Neonatal arterial blood gas pH < 7.2 or a base deficit of at least 12 mmol/lit with in the first hour of life, fetal distress, thick, meconium stained amniotic fluid and resuscitation for more than 1 minute with positive pressure ventilation. Babies with major congenital malformations, preterm and born through general anesthesia and Magnesium sulphate for Eclampsia were excluded from the study. Written informed consent was obtained from the parents before the commencement of study. Detailed antenatal, natal and postnatal history was obtained to elicit evidence of perinatal asphyxia and clinical examination of the newborn was performed to assess the gestational age (as per New Ballard score) and following which detailed systemic examinations was conducted. The clinical severity and progress of asphyxial insult was evaluated on a Classification of HIE (Sarnats and Sarnats).

Relevant investigations were carried out investigations as and when required: like Arterial Blood Gas, Nucleated RBC, Haemoglobin, Total Leucocyte Count, Differential Leucocyte Count, Platelet and Reticulocyte count, peripheral smear for General Blood Picture, blood urea, serum electrolyte, serum creatinine, sepsis, screen (absolute neutrophil count, band cell ratio, micro ESR, CRP) and blood culture and sensitivity. Cytological and biochemical evaluation of cerebrospinal fluid including culture and sensitivity was done. For Nucleated RBC count (case and control group), immediately after delivery, 1 mL of umbilical cord venous blood was collected in a tube containing 1.5 mg ethylenediaminetetra acetic acid (EDTA) and cord venous blood was dispatched to central pathology laboratory. Thin blood film was prepared from umbilical blood. The film was stained by Leishman’s stain and nRBC/ 100 WBC was counted under pathologist’s supervision.

The study was started after the approval of the Institutional Ethical Review Board. A written informed consent was obtained from each parent of study subjects. The participants had option to withdraw from the study anytime during their hospital stay.

Data were analyzed using the SPSS version 20.0. Chi-square and Fisher Exact tests were applied to compare the data of proportions and Student’s t-test was used for quantitative variables. A p-value of less than 0.05 was considered as statistically significant.

Results

Among case group, there were 52 (63.4%) males and 30 (36.5%) females and in the control group there were 54 (65.8%) males and 28 (34.1%) females. Mean birth weight was 2.90±0.32 and 2.90±0.31 in case and control group respectively. Fifty five (67.0%) neonates were delivered by spontaneous vaginal delivery while 27 (32.9%) were delivered by caesarean section in cases group and in control group 51 (62.1%) born through spontaneous vaginal delivery, while 31 (39.0%) neonates were delivered by caesarean section. In the present study 59 neonates were found to have NRBC level ≥10/100WBC, out of which 58 (70.7%) were cases and 1(1.2%) was a control. The number of neonates with NRBC levels ≥10/100WBC was significantly more in cases when compared to controls with p-value <0.001, as shown in Table 1.

While comparing the correlation between NRBCs and Apgar score it was found that NRBCs count of ≥10/100WBC were seen more in the newborn who had low 5 min Apgar score and this difference was statistically significant (p<0.001), as shown in table 2.

In the present study 18 (30.5%) newborn developed severe HIE who had NRBCs count ≥10/100WBC. Similarly none of the newborn developed severe HIE, who had NRBCs count <10/100WBC. Severity of mild HIE 10(16.9%) and moderate HIE 29(49.2%) were more in the newborn who had NRBC count ≥10/100WBC, in comparison to the newborn who had NRBCs count <10/100WBC. Interestingly, 2(3.4%) neonates of case group were without HIE had a NRBCs count ≥10/100WBC and 88(83.8%) had NRBCs count <10/100WBC. The relationship between cut-off NRBCs level of 10/100WBC with the severity of HIE was found to be significant (p<0.001), as shown in table 3.

The cut-off NRBC value of ≥10/100WBC has 8.67% sensitivity with a specificity of 97.75. NRBC has a positive predictive value of 96.12% with a negative predictive value of 84.47%, as shown in Table 4.

In the present study significance and sensitive area for ROC curve is 0.875 which is statistically significant p-value = <0.001. The ROC curve calculated with cut-off NRBC value of ≥10/100WBC.
Fig 1: Nucleated red blood cells as seen in the peripheral smear of cord blood

Table 1: Nucleated RBC in asphyxiated and non-asphyxiated newborn

<table>
<thead>
<tr>
<th>NRBC cut-off /100WBC</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Total (%)</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI for OR</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>24 (29.2)</td>
<td>81 (49.3)</td>
<td>105 (64.0)</td>
<td>&lt;0.001*</td>
<td>195.75</td>
<td>25.74-1488.35</td>
<td>S</td>
</tr>
<tr>
<td>≥10</td>
<td>58 (70.7)</td>
<td>1 (1.2)</td>
<td>59 (36.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82 (50)</td>
<td>82 (50)</td>
<td>164 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>12</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQR#</td>
<td>6, 13</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mann Whitney u test, S = significant, CI = confidence interval, OR= Odds ratio

Table 2: The association between Nucleated RBC with Apgar score at 5 min

<table>
<thead>
<tr>
<th>NRBC cut-off 100WBC</th>
<th>Apgar score</th>
<th>Total (%)</th>
<th>p-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤3 (%)</td>
<td>4-6 (%)</td>
<td>≥7 (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>0 (0)</td>
<td>26 (24.8)</td>
<td>81 (77.2)</td>
<td>105 (64.0)</td>
</tr>
<tr>
<td>≥10</td>
<td>6 (10.2)</td>
<td>52 (88.1)</td>
<td>1 (1.7)</td>
<td>59 (36.0)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (3.7)</td>
<td>76 (46.3)</td>
<td>82 (50.0)</td>
<td>164 (100)</td>
</tr>
</tbody>
</table>

*Chi square test, S = significant
in normal term newborns13,14. However Phelan et al studies, the range of NRBC counts was 7.56 to 8.55 were very similar to our study. According to the other with low Apgar score and different HIE were 10 (16.9%), 29 (49.2%) and 18 (30.5%) respectively. It indicates high NRBCs count is strongly associated with hypoxic ischaemic encephalopathy and it was statistically also significant in our study (p-value = <0.001). This association was also compared with other studies, according to Tungalag12 and Saracoglu et al13, there was a significant relationship between NRBC count and the degree of encephalopathy and this relationship was also statistically significant (p<0.001)12,13. Manjusa G et al17 also evaluated the relationship between HIE staging and nucleated RBC and they found that higher the HIE staging, higher the mean NRBC/100 WBC count. In their study severe HIE was 39.9%, moderate HIE was 45.2% and mild HIE was 18.2%, which is very similar to our study17. Neonates diagnosed with HIE were found to have higher NRBC counts, when compared with normal infants. NRBC count is significantly related to the grading of encephalopathy. Some other authors also evaluated the relation between the severity of asphyxia and cord NRBC count and the relationship was found to be significant13,16,18.

To determine the significance of mentioned NRBC level in perinatal asphyxia can be seen from the results of ROC curve test. In the present study significance and sensitive area for ROC curve is 0.875 which is statistically significant with p-value = <0.001 and it is very similar with the study done by Tungalag et al12, in which they found ROC area = 0.75 with p-value of <0.001.

In our study the cut-off NRBC value of ≥10/100WBC had sensitivity of 70.30%, specificity of 98.78%, positive predictive value of 98.31% and negative predictive value of 77.14%. According to Manjusa G et al17 study NRBCs count had a sensitivity of 94.0%, specificity of 81.3%, positive predictive value 98.0% and negative predictive value of 98.0%. In contrast Hassan B et al19 found NRBCs count had a sensitivity of 81.3% and specificity of 94.4%, which were very similar to our study. So it has been found that this simple test can be helpful in the rapid assessment of perinatal asphyxia.

### Table 3: The association between NRBC and severity of HIE

<table>
<thead>
<tr>
<th>NRBC cut-off</th>
<th>HIE</th>
<th>No HIE (%)</th>
<th>Mild (%)</th>
<th>Moderate (%)</th>
<th>Severe (%)</th>
<th>Total (%)</th>
<th>p-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10/100WBC</td>
<td>88 (83.8%)</td>
<td>15 (14.3%)</td>
<td>2 (1.9%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>105 (64%)</td>
<td>&lt;0.001*</td>
<td>S</td>
</tr>
<tr>
<td>≥10/100WBC</td>
<td>2 (3.4%)</td>
<td>10 (16.9%)</td>
<td>29 (49.2%)</td>
<td>18 (30.5%)</td>
<td></td>
<td>59 (36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90 (54.9%)</td>
<td>25 (15.2%)</td>
<td>31 (18.9%)</td>
<td>18 (11%)</td>
<td></td>
<td>164 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*chi square, S = significant

### Table 4: Sensitivity, specificity and predictive values of NRBC

<table>
<thead>
<tr>
<th>Category</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRBC</td>
<td>≥10</td>
<td>70.30%</td>
<td>98.78%</td>
<td>98.31%</td>
<td>77.14%</td>
</tr>
</tbody>
</table>

### Discussion

Newborn with perinatal asphyxia adjust to maintain adequate oxygen supply to central organs, aerobic metabolism is supplemented by anaerobic metabolism of glucose and glycogen to maintain cell and organ function and this anaerobic metabolism produces lactic acid and releases hydrogen ions, which leads to fall in pH, rise in the base deficit of the extracellular fluid and glucose store will be reduced in this process. The hypoxic event also induces a compensatory response in the form of exaggerated erythropoesis, resulting in the release of immature red blood cells (NRBCs) into the fetal circulation.

In the present study 59 neonates were found to have NRBC level ≥10/100WBC, out of which 58 (70.7%) were cases and 1 (1.2%) was a control. The median NRBCs count was 12 and 2.5 for cases and control groups respectively. The number of neonates with NRBC count of ≥10/100WBC was significantly more in cases when compared to control group (p-value = <0.001). Similar observation was noticed by Tungalag et al12 the mean NRBCs count was 11.36±10.7 in case group and 4.83±3.01 in control group, these finding were very similar to our study. According to the other studies, the range of NRBC counts was 7.56 to 8.55 in normal term newborns13,14. However Phelan et al found the mean NRBC of 3.4 per 100 WBCs in non-asphyxiated neonates and very high value of 34.5 in asphyxiated neonates15,16. Hence it shows that NRBC count increases in the asphyxiated newborn compare to non asphyxiated newborn.

We had also seen association of NRBCs counts with the severity of disease. We found that newborn with NRBCs counts of ≥10/100WBC was associated with low Apgar score and different grades of HIE. In the present study mild HIE, moderate HIE and severe HIE were 10 (16.9%), 29 (49.2%) and 18 (30.5%) respectively. It indicates high NRBCs count is strongly associated with hypoxic ischaemic encephalopathy and it was statistically also significant in our study (p-value = <0.001).
Conclusion

NRBC counts helps to differentiate asphyxiated from non-asphyxiated neonates. Neonates with NRBC levels ≥10/100WBC was significantly more in cases when compared to controls with p-value <0.001. The relationship of NRBCs and the severity of HIE was also found to be statistically significant (p-value <0.001). Similarly NRBCs count of ≥10/100WBC were seen more in the newborn who had low 5 min Apgar score and reference was also statistically significant (p-value <0.001). Hence the cord blood NRBCs/100 WBCs has a potential of being used as an early marker for determining the severity and predicting the outcome of perinatal asphyxia. In resource poor settings these bedside diagnostic tests are having high specificity and sensitivity with low cost and good feasibility. Hence, these markers can be very useful to differentiate HIE newborns from non-HIE newborns which will help in appropriate management and better outcome of these newborns.

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Conflict of Interest: Nil
Permission from IRB: Yes

References