Original Research Article

Cord blood and peripheral nucleated red blood cells levels in term asphyxiated and non-asphyxiated newborns and neonatal outcome: a comparative study

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ABSTRACT

Background: Perinatal asphyxia can cause severe hypoxic-ischemic damages in the organs and causes severe long-term consequences. It has recently been suggested that the increase in NRBC count in the umbilical vein of newborns can be considered a sign of birth asphyxia. The aim of the study was to evaluate the role of cord blood nucleated RBC count as a marker for presence and severity of birth asphyxia.

Methods: This prospective observational comparative study was conducted among 30 asphyxiated (case) and 30 non-asphyxiated newborns (control) in Swami Dayanand Hospital. Number of nucleated red blood cells in cord blood and peripheral blood were counted per 100 leukocytes in smears in cases and control. Maternal parameters and neonate characteristics were recorded in a proforma.

Results: NRBC count increased in cord blood during the first few hours of life in perinatal asphyxia (10.79±9.58) as compared with the healthy control subjects (4.03±2.87, p=0.002). Significant positive correlation of C-NRBC and P-NRBC with NICU stay (r=0.921, p<0.0001 and r=0.698, p<0.0001 respectively). Nucleated RBC count more than 18 can be used as predictor of abnormal neurological outcome with sensitivity 100% and specificity of 93.1%. The cord blood NRBC >3/100 WBC has sensitivity of 92% and specificity of 77.8% in predicting birth asphyxia.

Conclusions: Nucleated RBC count can be used as surrogate marker for birth asphyxia. It has significant positive correlation with severity of hypoxic ischemic encephalopathy, duration of NICU stay and duration of O₂ requirement.

Keywords: Cord blood, New born, Nucleated red blood cell, Perinatal asphyxia

INTRODUCTION

Birth asphyxia is the insult to the foetus or newborn, due to severe paucity of oxygen or lack of perfusion to various vital organs. Perinatal asphyxia can cause severe hypoxic-ischemic damages in the organs of neonates and causes severe long-term consequences or fatal complications. Despite, major advances in foetal surveillance technology; asphyxia is still one of the most significant causes of mortality and long-term morbidity in neonates and no single marker of perinatal asphyxia has shown good predictive efficiency. So, predicting the prognosis of asphyxia should be given highest priority. It has recently been suggested that the increase in NRBC count in the umbilical vein of newborns can be considered a sign of birth asphyxia.¹ In healthy newborns, NRBC count is reduced by half at 12 hours after birth, and by the third or fourth day of birth, NRBCs are not seen in the blood circulation.²-⁴

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 Increase in NRBC count is often
due to prematurity, ABO or Rh blood incompatibility, increase in hematopoiesis followed by chronic diseases in mother, maternal diabetes, preeclampsia, fetal anemia, intrauterine infections, chorioamnionitis and acute or chronic asphyxia.  

The number of NRBC/100 WBC is variable but is rarely greater than 10 in normal neonates. Its variation in neonates suffering from birth asphyxia is studied and compared to the severity of clinical outcome in these children. The aim of the study was to evaluate the role of cord blood nucleated RBC count as a marker for presence and severity of birth asphyxia.

METHODS

Study area
All the normal newborns and newborns suffering from birth asphyxia delivered in Swami Dayanand Hospital, Delhi.

Type of study
The type of study was prospective observational comparative study.

Study duration
The study duration was March 2018 to November 2018.

Participants
Cases
Newborns suffering from birth asphyxia in Swami Dayanand Hospital were considered cases.

Controls
Normal newborns delivered in Swami Dayanand Hospital were in controls.

Sample size
The study of Sylvia et al observed that mean NRBC level in cases and control groups was 17.43±19.86/100 WBCs and 2.97±4.79/100 WBCs respectively. Taking these values as reference, the minimum required sample size with 90% power of study and 5% level of significance was found to be 21 patients in each study group with using below mentioned formula.

\[
N \geq \frac{2(\text{SD})^2(Z_{a} + Z_{b})^2}{(\text{Mean difference})^2}
\]

Taking attrition rate to be 15%, calculated sample size was 25. To reduce margin of error, total sample size taken was 60 (30 patients per group).

Inclusion criteria

Study group
All term neonates born with perinatal asphyxia which is defined as any of the following: (a) signs of fetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability or Thick, meconium stained amniotic fluid; (b) babies born with respiratory depression, hypotonia, or bradycardia; and (c) need for resuscitation for more than 1 minute with positive pressure ventilation immediately after birth.

Control
Term appropriate for gestation newborns, with an uneventful postnatal clinical course with gestational age more than equals to 37 weeks gestation- (a) normal fetal heart rate (FHR) during intrapartum period; (b) clear amniotic fluid; and (c) neurologically normal newborns

Exclusion criteria
Patients with following criteria’s were not included- (a) pre-term neonates; (b) with Rh-incompatibility; (c) infant of diabetic mother; (d) cyanotic congenital heart disease; (e) maternal fever >100.4°F; (f) chorioamnionitis; (g) congenital heart block; and (d) narcotic exposure in pregnancy.

Total 30 asphyxiated and 30 non-asphyxiated newborns born in Swami Dayanand Hospital were included in the study. Newborns who qualify were subjected to a thorough clinical examination and investigations. An informed consent was taken from the parents before performing the study after explaining in detail about the methods and procedures involved in the study in their vernacular language. 2 ml cord blood sample was drawn immediately after birth in an EDTA (ethylene di-amine tetra acetic acid) vial. The blood sample was analyzed for hemoglobin percent, total leukocyte count, differential leukocyte count, hematocrit and erythrocyte sedimentation rate.

Blood smear was prepared with wright’s stain and manual differential count was done to count NRBCs. Number of nucleated red blood cells were counted per 100 leukocytes in smears and were reported as ‘number of NRBC/100 WBC’.

1 ml of venous blood sample was taken at 72 hours of age of asphyxiated newborns during their NICU stay. These reports were observed and then analyzed for immediate correlation with different degrees of asphyxia. During the same period, cord blood samples of non-asphyxiated newborn which act as controls were taken and subjected to similar hematological evaluation. Details of the maternal parameters like age, blood group, hemoglobin level, past obstetric history, present pregnancy, medical history, medications taken during pregnancy, details of labor and delivery were recorded in a proforma. Details of the baby
like date of birth, sex, gestational age, birth weight resuscitation (American Academy of Pediatrics guidelines) and examination details were recorded and analysed.

The data was entered in MS excel spreadsheet and analysis was done using Epi info version 7. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean±SD and median. Normality of data were tested by Kolmogorov Smirnov test. If the normality was rejected then non-parametric test were used. Quantitative variables were compared using Unpaired t-test/Mann Whitney test (when the data sets were not normally distributed) between the two groups. Qualitative variables were compared using Chi square test/Fisher’s exact test. A p value of <0.05 were considered statistically significant.

RESULTS

Table 1 shows baseline characteristics of the mothers and neonates. The predominant age group of mothers in both the cases and controls was 19-25 years, i.e.; 65% in case group and 76% in controls, which was statistically insignificant. Almost an equal proportion i.e. 58% cases and 30% controls were born to primigravidas. The mean gestational age in cases (38.87±1.01) was lower than the gestational age of controls (39.56±0.85). There were more caesarean sections in case group (53%) than vaginal deliveries as compared to control group which had more number of vaginal deliveries (80%). This was statistically significant (p=0.001). Table 1 and Figure 4 shows mode of delivery amongst study groups. Cases and controls were similar in terms of sex distribution. The mean±SD of birth weight in cases (2.89±0.36) was lower than the mean birth weight (2.96±0.35) in controls.

Hemoglobin and total leukocyte count (TLC) in case group (15.91±1.98 and 15722.65±5811.08, respectively) was higher than the control group (14.76±1.49 and 13233±3420.74) and was statistically significant (p=0.012 and p=0.04) respectively. Mean C-NRBC was 10.79±9.58 in cases and 4.03±2.87 in control. The difference was statistically significant as p=0.002. Cut-off value for C-NRBC as >3/100 WBC had a sensitivity of 92% and specificity of 77.8% in predicting birth asphyxia. In our study, not all newborns were resuscitated with PPV and 9 out of 34 newborns were managed by initial steps of resuscitation (Table 2).

Mean cord blood and peripheral blood NRBCs were significantly higher in the newborns requiring positive pressure ventilation (PPV), than in newborns not requiring PPV in case group (p=0.0005 and p=0.027 respectively), as shown in Figure 1.

In cases there were 38%, 26%, 15% newborns with HIE 1, HIE 2, HIE 3 respectively. Mean value of C-NRBC showed an increasing trend with increase in HIE stage (from 2.57±1.72 at stage 0 to 28±7.58 at stage 3). Mean value of P-NRBC also showed an increasing trend with increase in HIE stage (from 1±1.15 at stage 0 to 4.25±1.89 at stage 3) and was statistically significant (p<0.0001 and p=0.008) respectively (Figure 2).

Table 3 shows significant positive correlation between C-NRBC count with NICU stay and duration of O2 requirement with r value=0.921 each and was statistically significant (p<0.0001). Similarly, significant moderate positive correlation also existed between NICU stay and duration of O2 requirement with P-NRBC (r=0.698) and (r=0.665) respectively. Both were statistically significant (p<0.0001 for each).

Out of 34 cases, one newborn died on day 2 and 4 newborns (12%) were found to be neurologically abnormal at 45 days of life by Amiel-Tison method of examination. In neurologically abnormal babies, NRBC counts were significantly higher for both cord blood (23±2.45) and peripheral blood (4±2.16) as compared to normal babies. This was statistically significant p=0.003 and p=0.021 respectively (Figure 3).

On performing univariate logistic regression, C-NRBC was found to be a significant risk factor for predicting abnormal outcome and P-NRBC was found to be borderline significant. With the increase in C-NRBC by 0.1 units, the chances of abnormal outcome got significantly increased by 3.4%. Similarly, with the increase in P-NRBC by 0.1 units, the chances of abnormal outcome got increased by 20.7% which was borderline significant (Table 4). On ROC analysis, cut-off value of cord blood NRBC was >18 with sensitivity 100% and specificity 93.1% and for peripheral blood NRBC the cut off value was >1 with sensitivity and specificity 100% and 51.72% respectively (Table 5).

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Table 1: Baseline characteristics of the mothers and neonates.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Case (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-25</td>
<td>22 (65)</td>
<td>23 (76)</td>
</tr>
<tr>
<td>26-32</td>
<td>12 (35)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>33-38</td>
<td>0 (0)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primi</td>
<td>20 (58)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>G2P1</td>
<td>11 (32)</td>
<td>19 (63)</td>
</tr>
<tr>
<td>G3P2</td>
<td>3 (10)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Gestational age (Mean±SD)</td>
<td>38.87±1.01</td>
<td>39.56±0.85</td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instrumental</td>
<td>4 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>LSCS</td>
<td>18 (53)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Vaginal</td>
<td>12 (35)</td>
<td>24 (80)</td>
</tr>
<tr>
<td><strong>Neonates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>M: F 1.1: 1</td>
<td>1: 1</td>
</tr>
<tr>
<td>Birth weight</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.89±0.36</td>
<td>2.96±0.35</td>
</tr>
</tbody>
</table>

Table 2: Hematological analysis of cases and control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (mean±SD)</th>
<th>Controls (mean±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>15.91±1.98</td>
<td>14.76±1.49</td>
<td>0.012</td>
</tr>
<tr>
<td>TLC</td>
<td>15722.65±5811.08</td>
<td>13233±3420.74</td>
<td>0.04</td>
</tr>
<tr>
<td>C-NRBC</td>
<td>10.79±9.58</td>
<td>4.03±2.87</td>
<td>0.002</td>
</tr>
<tr>
<td>P-NRBC</td>
<td>1.91±1.55</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 3: Correlation between duration of NICU stay and O₂ requirement.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>NICU stay</th>
<th>O₂ requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-NRBC</td>
<td>Correlation coefficient 0.921</td>
<td>0.922</td>
</tr>
<tr>
<td></td>
<td>P value &lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N 33</td>
<td>33</td>
</tr>
<tr>
<td>P-NRBC</td>
<td>Correlation coefficient 0.698</td>
<td>0.665</td>
</tr>
<tr>
<td></td>
<td>P value &lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N 33</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 4: Univariate logistic regression for predicting abnormal neurological outcome.

<table>
<thead>
<tr>
<th>NRBC</th>
<th>B</th>
<th>SE</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% CI for Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-NRBC</td>
<td>0.293</td>
<td>0.131</td>
<td>0.026</td>
<td>1.340</td>
<td>1.036-1.732</td>
</tr>
<tr>
<td>PB-NRBC</td>
<td>1.121</td>
<td>0.572</td>
<td>0.050</td>
<td>3.068</td>
<td>0.999-9.415</td>
</tr>
</tbody>
</table>

Figure 1: Relation of NRBC in cord blood and peripheral blood with requirement of PPV.

Figure 1: Relation of NRBC in cord blood and peripheral blood with requirement of PPV.
Table 5: ROC analysis of mean nucleated NRBC and abnormal outcome.

<table>
<thead>
<tr>
<th>NRBC</th>
<th>Area under ROC curve (AUC)</th>
<th>P value</th>
<th>Cut off</th>
<th>Sn</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-NRBC</td>
<td>0.961</td>
<td>&lt;0.0001</td>
<td>&gt;18</td>
<td>100</td>
<td>93.1</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>PB-NRBC</td>
<td>0.853</td>
<td>0.0002</td>
<td>&gt;1</td>
<td>100</td>
<td>51.72</td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 2: Relationship of HIE stage with mean NRBC count.

Figure 3: Mean NRBC value in normal and abnormal newborns.

Figure 4: ROC curve for neurological abnormal newborns with cut off values.
DISCUSSION

NRBC as a predictor of birth asphyxia

In the present study, NRBC count/100 WBC increased in cord blood during the first few hours of life in perinatal asphyxia (10.79±9.58) as compared with the healthy control subjects (4.03±2.87, p=0.002).

Several studies have reported an increased NRBC in neonatal cord blood following perinatal asphyxia. Studies done by Ganta et al and Hemlatha et al, reported 11.6±5.736 and 15.7±3.58 NRBC respectively, which was nearly similar to present study observations. The mean cord blood NRBC observed by Kumar et al and Khurana et al was 20.97±8.17 and 21.40±20.31 respectively. Values in the latter two studies were higher than the present study because they included newborns with Apgar score <7 at 5 min and the newborns that required positive pressure ventilation at birth.

The results of the present showed the cut-off value for NRBC as >3/100 WBC with a sensitivity of 92% and specificity of 77.8% in predicting birth asphyxia. Boskabadi et al. reported a cut-off value for NRBC as >70/mm³ with sensitivity of 83.4% and specificity of 73.5% in predicting perinatal asphyxia which cannot be compared with values in per 100 WBC in present study. Srivastava et al reported a cut off value for NRBC as ≥10 as a predictor of birth asphyxia with sensitivity of 88.75% and specificity of 100%. In the present study, not all newborns (9 out of 34) were resuscitated with PPV. Whereas Srivastava et al enrolled babies who required either basic or advanced resuscitation.

NRBC and HIE

In present study, neonates diagnosed with HIE were found to have higher NRBC counts and counts were also elevated in infants who subsequently died when compared to those who survived.

The mean NRBCs with no HIE, HIE-1, HIE-2, HIE-3 were 2.57±1.72, 6.46±4.29, 13.89±5.9, 28±7.58 respectively. Relatively closer values in each HIE subgroups were observed by Ganta et al with the mean NRBC counts of 5.91, 12.50, 17.67, 29.02 respectively in No HIE, HIE 1, HIE 2 and HIE 3. Similarly, Boskabadi et al, found the mean NRBC counts as 9.75, 11.94, 21.08 and 29.18 in No HIE, HIE 1, HIE 2, HIE 3 respectively.

Correlation of NRBC with O₂ requirements, duration of NICU stay

In the present study, significant strong positive correlation existed between NICU stay and C-NRBC with r=0.921, p<0.0001. Significant moderate positive correlation existed between NICU stay and P-NRBC with r=0.698, p<0.0001. It means more the value of C-NRBC and P-NRBC, longer will be NICU stay. Similarly, significant strong positive correlation (r=0.922, p<0.001) and moderate positive correlation (r=0.665, p<0.001) existed between O₂ requirement and C-NRBC and P-NRBC respectively. It means more the value of C-NRBC and P-NRBC, more will be duration of O₂ requirement. While reviewing the literature, none of the studies were found showing any correlation between C-NRBC and P-NRBC with the duration of NICU stay and the duration of O₂ requirement in asphyxiated newborns to the best of our knowledge.

Correlation of NRBC with neurological outcome

In the present study, 12% newborn in case group (4/34) were neurologically abnormal at 45 days of life. Mean NRBC counts were significantly higher for both cord blood and peripheral blood in abnormal babies (23±4.25, 4±2.16 respectively) as compared to normal babies (1.62±1.24, 8.1±±4.96 respectively). P value for cord and peripheral blood were 0.003 and 0.021 respectively. It can be thus concluded that the higher P-NRBC leads to abnormal outcome as compared to lower P-NRBC.

These correlations are in accordance with study done by Kumar et al who followed up newborns at 3 month of age and found that higher the grade of HIE, greater was neurological impairment. They found statistically significant difference in both motor and mental neurological impairment in newborns at 3 months of age when cord blood NRBC values were >20 (p<0.006). Rai et al found higher mean P-NRBC of 7.9±±6.0 and 9.8±±8.9 in babies with abnormal neurological status at discharge and with sequelae at 6 months respectively, which was highly statistically significant than normal status at discharge and 6 months (p<0.05).

Limitations

Only term newborns were included in the study. Therefore, results cannot be generalized to whole neonatal population. Babies were followed only up at 45 days of life. Therefore, the association of nucleated RBC count with long term neurological outcome could not be determined. This study did not correlate NRBC count with cord blood pH which is reported to be a reliable marker of perinatal asphyxia.

CONCLUSION

Nucleated red blood cell count can be used as surrogate marker for birth asphyxia. It has significant positive correlation with severity of hypoxic ischemic encephalopathy, duration of NICU stay and duration of O₂ requirement.

Nucleated red blood cell count more than 18 can be used as predictor of abnormal neurological outcome with sensitivity 100% and specificity of 93.1%. The cord blood NRBC >3/100 WBC has sensitivity of 92% and specificity of 77.8% in predicting birth asphyxia.
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