

Study of nucleated RBC count as a marker of severity of perinatal asphyxia in newborns - a case control study

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Abstract

Introduction: This study was done to estimate the nucleated red blood cell count (NRBCs) in normal and asphyxiated babies and find their correlation with severity of birth asphyxia. **Materials and Methods:** About 50 normal newborns as control and 50 newborns with perinatal asphyxia as cases were considered. At birth 2 mL of venous blood was collected in both cases and control groups. nRBC count per 100 white blood cells (WBC) was done at admission. Clinical assessments in terms of neurologic status at birth, 24 hours after birth and every day thereafter till discharge/death was done. Cord blood nRBC/100 WBC was correlated with stages of HIE during hospital stay. Neonates were monitored for adverse outcome such as tone abnormalities, feeding difficulty, refractory seizures and death. Controls were followed up in the same manner. **Results:** Among 50 cases, 24 had no hypoxic ischaemic encephalopathy (HIE), 17 had stage 1 HIE, 6 had stage 2 and 3 newborns had stage 3 HIE. The mean APGAR score in cases was 5.34 ± 1.19 whereas for the control group it was 8.12 ± 0.77 with p value of 0.001 which is statistically significant. The mean nRBC in newborns with APGAR score of 3, 4, 5, 6 were 35.5, 19.9, 20.6 and 12 respectively. The low APGAR scores showed high nRBCs. Mean nRBCs for HIE stage 1, 2, 3 was 19.1, 31.0, and 54.3 respectively. High nRBC count correlated with increasing severity of birth asphyxia. **Conclusion:** nRBC count is an easy, simple and a reliable test to assess the severity of birth asphyxia in resource poor settings.

Key words: Perinatal asphyxia nucleated RBC, APGAR score.

Introduction

Perinatal asphyxia (derived from the Greek word *asphyxios* meaning born without an evident pulse) is one of the most important causes of fetal distress [1]. According to WHO estimates, around 3% of approximately 120 million infants born every year in developing countries develop birth asphyxia [2]. In India, between 250,000-350,000 infants die each year due to birth asphyxia 2.

Asphyxia may occur in utero, at birth or in the postnatal period. World Health Organization (WHO) 2 has defined perinatal asphyxia as a "failure to initiate and sustain breathing at birth". Data from National Neonatal Perinatal database 3 suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India 3 and defines moderate asphyxia as slow gasping

breathing or an APGAR score of 4 - 6 at 1 min of age, and severe asphyxia was defined as no breathing or an APGAR score of 0-3 at 1 minute of age [3]. Perinatal 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 [4]. Perinatal asphyxia results in hypoxic injury to various organs including kidneys, lungs and liver but the most serious effects are seen on the central nervous system [1,5]. Hypoxic ischemic encephalopathy (HIE) refers to the CNS dysfunction associated with perinatal asphyxia. Classification of HIE (Stage I, Stage II and Stage III) in term neonates was proposed by Sarnat and Sarnat[6]. No single parameter can define perinatal asphyxia, rather a combination of parameters like fetal distress, meconium-stained liquor, low APGAR score, umbilical cord blood pH and clinical features of hypoxic ischemic encephalopathy (HIE) can predict it 4. Recent studies on hematological variations in

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asphyxiated neonates as a predictor of neonatal asphyxia have suggested that number of nucleated red blood cells (NRBCs) in cord blood of asphyxiated neonates help in identifying birth asphyxia [4]. 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 levels of nRBC may be correlated with the presence of perinatal asphyxia [7]. The number of nRBC/100 white blood cells (WBC) is quite variable but is rarely >104. The instances, where number of nRBCs exceed >10/100 WBC are prematurity, ABO or Rh incompatibility, maternal diabetes, intrauterine growth retardation [8], acute asphyxia, congenital infection, cyanotic heart disease, pre-eclampsia, maternal smoking, and chorio-amnionitis. Considering the hematopoietic response to hypoxia in utero, the elevated NRBC/100 WBC count is being hailed as the marker for not only perinatal asphyxia but also to predict the chances of the neonates developing neurological sequelae [4].

Objectives of the study- The aim of the study was to estimate the nucleated red blood cell count (NRBCs) in normal and asphyxiated babies and its correlation with severity of birth asphyxia and immediate outcome of such babies.

Materials and Methods

Study design: Case control clinical study.

Study center: Dr. B R Ambedkar Medical College and Hospital.

Sample Size: Sample size was based on inclusion and exclusion criteria. 50 normal newborns as control and 50 newborns with perinatal asphyxia as cases were included in the study.

Statistical analysis was done using SPSS version 20 software. 't' test of significance was used to compare mean APGAR scores.

Inclusion Criteria

Cases -Term newborns (>37 weeks to 42 weeks of gestation) with perinatal asphyxia

- 1) APGAR score < 7 at 5 minutes of life.
- 2) Thick, meconium stained amniotic fluid and respiratory depression, hypotonia or bradycardia.

Results

The present study was done to find the correlation of severity of birth asphyxia with nucleated RBCs. About 50 normal newborns and 50 newborns with low APGAR score < 7 at 5 min of life were included in the study.

Among 50 cases, 24 had no HIE, 17 had stage 1 HIE, 6 had stage 2 and 3 newborns had stage 3 HIE.

- 3) Signs of fetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability).
- 4) A need for resuscitation for more than 1 minute with positive pressure ventilation and oxygen immediately after birth.
- 5) Heart rate of < 100 beats/min after 1 min of birth.

Control group included non-asphyxiated newborns (>37 to 42 weeks gestation)

- 1) Birth weight > 2,500 g
- 2) APGAR score \geq 7 at both 1 and 5 minutes
- 3) Normal intrapartum fetal heart rate (FHR) pattern
- 4) Clear amniotic fluid
- 5) Normal neurologic evaluation at birth.

Exclusion criteria: Preterm neonates, Infant of diabetic mother, newborns with CHD, RH incompatibility, chorio-amnionitis are excluded.

Method of Collection of Data: Term newborns (>37 weeks to 42 weeks of gestation) with perinatal asphyxia were included as cases. Control group included non-asphyxiated newborns (>37 to 42 weeks gestation), with birth weight > 2,500 g, and normal neurologic evaluation at birth, enrolled within 6 hours of birth. There was no randomization done. Newborns in the study period were taken in and classified as cases and controls depending on the inclusion and exclusion criteria. At admission 2 mL of venous blood was collected in EDTA tube in both cases and control groups and nRBC count per 100 white blood cells (WBC) was done. Clinical assessment of the neurologic status was done by 2 paediatricians not involved in the study and a neurologist who was unaware of the study. Neurological examination was done at birth, 24 hours after birth and every day thereafter till discharge/death, the grade of HIE (Stage I, Stage II or Stage III), the type of respiratory support needed, the presence of seizures, involvement of multi organ dysfunction, the time of establishment of direct breast feeds, and neurologic examination at discharge were done. Cord blood nRBC/100 WBC was correlated clinically with different stages of HIE during the hospital stay. Adverse outcome was implied if there was presence of at least one of the following condition such as death, hypertonicity or significant hypotonia, feeding difficulty, seizures resistant to phenobarbital. Controls were followed up in the same manner.

Table-1: Distribution of neonates according to APGAR score at 5 minutes.

Group	N	Median	Mean \pm SD	p-value
Cases	50	5	5.34 \pm 1.19	0.001 *
Control	50	8	8.12 \pm 0.77	
Total	100	7	6.73 \pm 1.72	

p value is statistically significant.

This table indicates distribution of neonates based on APGAR score at 5 min for study and control group. The mean APGAR score in cases was 5.34 \pm 1.19 whereas for the control group it was 8.12 \pm 0.77 with p value of 0.001 which is statistically significant.

Table-2: Correlation of APGAR score at 5 minute and mean nRBC count.

Apgar Score at 5min	N	MeannRBC \pm SD
3	4	35.5 \pm 18.0
4	7	19.9 \pm 11.1
5	17	20.6 \pm 12.4
6	12	12.0 \pm 12.3
7	22	7.0 \pm 1.9
8	20	7.6 \pm 1.7
9	18	7.6 \pm 2.2
Total	100	12.2 \pm 10.6

Table 2 indicates correlation of APGAR score at 5 min and nRBC count. The mean nRBC count in newborns with APGAR score of 3, 4, 5, 6 were 35.5, 19.9, 20.6 and 12 respectively. Babies with low APGAR scores had high nRBCs.

Table-3: Distribution of nRBCs according to HIE stages.

HIE	N	Mean \pm SD
No	24	6.9 \pm 1.7
stage1	17	19.1 \pm 5.3
stage2	6	31.0 \pm 8.7
stage3	3	54.3 \pm 4.7
Total	50	16.8 \pm 13.4

This table shows distribution of nRBCs according to the stages of HIE. Mean NRBCs for HIE stage 1, 2, 3 was 19.1, 31.0, and 54.3 respectively.

Table-4: Distribution of neonates according to nRBCs

nRBC	CasesN (%)	ControlN (%)	Total
\leq 10	24 (48)	48 (96)	72
11-20	12 (24)	2 (4)	14
21-30	7 (14)	0 (0)	7
>30	7 (14)	0 (0)	7
Total	50 (100)	50 (100)	100

This table shows the distribution of neonates according to nucleated RBCs. It was found that 96% of neonates in control group had nRBC count of $<10/100$ WBCs which is normal, whereas only 48% of cases had normal nRBC count. The remaining 52% of neonates among cases has nRBCs $>10/100$ WBCs which is considered significant.

Discussion

Perinatal asphyxia is a major cause of acute mortality and chronic neurologic disability amongst survivors [4]. Perinatal asphyxia results in hypoxic injury to various organs including kidneys, lungs and liver but the most serious effects are seen on the central nervous system. No single parameter can define perinatal asphyxia, rather a combination of parameters like fetal distress, meconium-stained liquor, low APGAR score, umbilical cord blood pH and clinical features of hypoxic ischemic encephalopathy (HIE) can predict it [4]. Recent studies have suggested that number of nucleated red blood cells (NRBCs) in cord blood of asphyxiated neonates help in identifying birth asphyxia [4,14]. The levels of nRBC may be correlated with the presence of perinatal asphyxia [7].

The number of nRBC/100 white blood cells (WBC) is quite variable but is rarely $>10/100$ WBCs [4]. Considering the hematopoietic response to hypoxia in utero the elevated nRBC/100 WBC count is being hailed as the marker for perinatal asphyxia and also to predict the chances of the neonates developing neurological sequelae [4]. It can be used as a prognostic marker to anticipate neonatal complications of severe birth asphyxia and to manage them effectively.

In our present study, the mean APGAR score at 5 minutes for cases was 5.34 ± 1.19 as compared to controls which was 8.12 ± 0.77 , with the p value of 0.001. This was comparable to the study done by Mohan et al [11] and Tae Hwan Kil et al [12].

Our study showed significant correlation between APGAR score at 5 minutes and nucleated RBC count as shown in table 2. The APGAR scores were inversely proportional to nRBC count. This was comparable to the study done by Ganta S.J et al [9] and Colaco et al [14].

The present study showed a strong correlation between the NRBCs and severity of birth asphyxia. The mean nRBC count was 19.1 in stage 1 HIE, 31 in stage 2 HIE and 54.3 in stage 3 HIE. Other studies have observed lower number of cord blood nRBCs in severe birth asphyxia compared to our study [9,10,13,17].

Meena P et al [15], in their study compared mean nucleated RBC count in neonates with and without birth

asphyxia. A statistically significant negative correlation of nRBC count was found with severity of birth asphyxia.

Mohanty et al [11], in their study showed mean nRBC of 15.8 in stage 1 HIE, 46.5 in stage 2 HIE and 77.1 in stage 3 HIE. This was comparable to our study except that mean nRBC in stage 3 HIE was higher.

Study conducted by Hermansen M.C et al [16], has concluded that an increase in nRBC counts are seen with acute and subacute asphyxia, the magnitude of the increase is a function of the severity and duration of asphyxia.

Hereby our study strongly supports that nRBC count is an easy, simple and a reliable test for severity of birth asphyxia.

Limitations of the study: Correlation of nRBC with blood gas analysis was not done in our study. nRBCs were not correlated with complications of birth asphyxia.

What this study adds to the existing knowledge?

Combination of parameters such as fetal distress, low APGAR scores, cord blood pH and many others are used to predict severity of birth asphyxia.

But our study suggest that nRBC count can be used as an early and an alternative marker to predict the severity of birth asphyxia.

Conclusion

nRBC count is an easy, simple and a reliable marker for severity of birth asphyxia in a resource poor settings where facilities for ABG, cord pH analysis are not easily available.

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References

1. Steer PJ, Danielian P. Fetal distress in labour. In: James, Steer, Weiner, Gonik. High Risk Pregnancy. 3rd edition. WB Saunders; 2005:1450-1471

2. World Health Organization. Neonatal and Perinatal Mortality; Country, Regional and Global estimates, 2004; WHO, Geneva. 2006; 1-25.;
3. National Neonatal and Perinatal Database Report. 2002-2003;1-58.
4. Colacoa SM, Ahmed M, Kshirsagar VY, Bajpai R. Study of nucleated red blood cell counts in asphyxiated newborns and the fetal outcome. *IntJClinPediater*. 2014; 3(3):79-85.doi:http://dx.doi.org/10.14740/ijcp169w
5. IAP-NNF Guidelines on neonatal resuscitation; 2006; 137-140.
6. Sarnat HB, Sarnat MS. Neonatal Encephalopathy Following Fetal Distress. A Clinical and Electroencephalographic Study. *Arch Neurol*.1976; 33(10): 696-705.
7. Ghosh B, Mittal S, Kumar S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. *Int J Gynaecol Obstet*. 2003 Jun; 81(3):267-71.
8. Maier RF, Bohme K, Dudenhausen JW. Cord blood erythropoietin in relation to different markers of fetal hypoxia. *Obstet Gynecol*. 1993;81:575-580.
9. Ganta SJ, Kulkarni SR, Renuka DB. Study of cord blood nucleated RBC's as a marker for fetal asphyxia. *Int J Reprod Contracept Obstet Gynecol* 2017;6:658-62.DOI: <http://dx.doi.org/10.18203/23201770>. *ijrcog* 20170402.
10. Rai R, Tripathi G, Singh DK. Nucleated RBC Count as Predictor of Neurological Outcome in Perinatal Asphyxia. *Indian Pediatrics*. March 2014; 51
11. Mohanty AK, Das L, Pradhan S, Meher B, Beriha SS. Cord Blood Nucleated RBC as a Predictor of Perinatal Asphyxia, Severity and Outcome. *Ind J Clin Pra*. 2014; 24(10).
12. Kil TH, Han JY, Kim JB, Ko GO, Lee YH, Kim KY et al. A Study on the measurement of the nucleated red blood cell (nRBC) count based on birth weight and its correlation with perinatal prognosis in infants with very low birth weights. *Korean J Pediatr* 2011; 54(2): 69-78 . DOI: 10.3345/kjp.2011.54.2.69
13. Boskabadi H, Maamouri G, Sadeghain MH, Ghayour-Mobarhan M, Heidarzade M, Shakeri MT, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: a case-control study. *Arch Iran Med*. 2010;13(4):275-81.
14. Hanlon-Lundberg KM, Kirby RS. Nucleated red blood cells as a marker of acidemia in term neonates. *Am J Obstet Gynecol*. 1999;181:196-201.
15. Meena P et al. Correlation of nucleated red blood cells with severity of birth asphyxia and its immediate outcome in term newborns. *Int J ContempPediater*. 2016 Aug; 3(3): 841-846. DOI:<http://dx.doi.org/10.18203/2349-3291>. *ijep* 20161430
16. Hermansen MC. Nucleated red blood cells in the fetus and newborn. *Arch Dis Child Fetal and Neonatal Ed*. 2001; 84(3):F211-5.
17. Mamta Malik, Ritu Mital, Vijay Jaiswal, Nisha P. Malik, Vikas Agarwal, Amit Upadhyay. Correlation of acidosis with blood markers (LDH and nucleated RBCs/100 WBC) and with mortality and neuro developmental outcome in neonates with perinatal asphyxia. *International Journal of Contemporary Medical Research* 2016;3(4):938-942.

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