CORD BLOOD NUCLEATED RED BLOOD CELL COUNT: A SIMPLE BEDSIDE TEST OF PERINATAL ASPHYXIA AND ITS CORRELATION WITH IMMEDIATE OUTCOME

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ABSTRACT

BACKGROUND

Asphyxia is a leading cause of foetal neonatal mortality and morbidity. Nucleated red blood cell count (NRBC) produced as compensatory response to asphyxia in foetus and NRBC level can be correlated to asphyxia. Because the present indices are unhelpful in the diagnosis and prediction of the severity of asphyxia, we wished to investigate the relationship between the nucleated RBC count and the severity & immediate outcome of perinatal asphyxia.

METHOD

This prospective comparative study was conducted in maternity ward of Obstetrics & Gynaecology Department and Paediatric Department of GSVM Medical College, Kanpur (Central UP), from January 2014 to September 2014. Newborns of term gestation were selected after satisfying inclusion criteria and were divided in 2 groups. The control group consisted 60 normal newborns and case group had 60 asphyxiated newborns. The cord blood was collected soon after birth, investigated for pH and making smears that were stained with Leishman's stain. NRBCs were counted against 100 WBCs. The statistical analysis was done using IMSTAT.

RESULTS

The mean NRBC count in the study group was 22.63±6.95 as compared to 4.75±2.04 in the control group (p=<0.0001). The NRBC count was significantly higher in low pH, neonates with low Apgar scores of < 3 at 1 minutes, newborns with HIE stage III & in neonates who were neurological abnormal at discharge (P=0.0001).

CONCLUSIONS

A simple, easy to do, cost effective bedside test, such as NRBC count at time of delivery is a good marker of perinatal asphyxia & its forthcoming immediate neurological outcome**.**

KEYWORDS

Asphyxia, Nucleated Red Blood Cell Count, Hypoxic Ischaemic Encephalopathy.

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INTRODUCTION: According to WHO estimates, around 3% of approximately 120 million infants born every year in developing countries develop birth asphyxia. Out of all the records available, 104 children die of birth asphyxia in an hour.,[1] that is approximately 8% of the total global paediatric mortality (age less than five years) making it very serious problem in developing countries**.** Perinatal asphyxia is an insult to the foetus or newborn due to lack of oxygen (hypoxia) and/or lack of perfusion (ischaemia) to the brain & also other organs. Diagnosis of Hypoxic Ischaemic 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.

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In asphyxiated neonates, there are many biochemical and haematological variations like acidosis, abnormal electroencephalogram, altered cerebral blood flow, hypoxia and hypercarbia, etc. The hypoxic event induces a compensatory response in the form of increased erythropoietin production & thereby exaggerated erythropoiesis, resulting in the release of immature red blood cells into the foetal circulation. Therefore, levels of NRBC may be correlated with the presence of perinatal asphyxia. It is also affected by factors such as prematurity, cyanotic congenital heart disease, foetal growth restriction, iso-immunisation, maternal tobacco use, maternal diabetes mellitus and chorioamnionitis (included as exclusion criteria). It is a simple test which can be done in a basic setup. The number of NRBC/100 WBCs is variable but is rarely >10 in normal neonates. We tried to investigate the relationship between the increase in NRBC count/100 WBCs with the severity of asphyxia & its immediate outcome in the newborns.

AIMS AND OBJECTIVE:

1) To study the cord blood nucleated RBC count (NRBC) at birth in babies with or without asphyxia.
2) To find out the correlation between NRBC count, clinical and biochemical markers of asphyxia and its immediate outcome of asphyxia.

MATERIAL AND METHOD: This prospective comparative study was conducted in maternity ward of Obstetrics & Gynaecology Department and NICU of Paediatric Department of GSVM Medical College, Kanpur (Central U.P.). All babies meeting inclusion criteria and exclusion criteria for study from January 2014 to September 2014 were included. A total of 120 term newborns were selected for the study, further divided in to case group of 60 asphyxiated newborns and 60 normal newborns in control group.

Inclusion Criteria: Presence of at least two of the criteria:

- Signs of foetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability).
- Thick meconium stained liquor.
- Apgar score <3 for longer than five minutes.
- Need for resuscitation for more than 1 minute with positive pressure ventilation or oxygen requirement after birth.
- Respiratory depression, seizures, hypotonia, and/or bradycardia.
- Blood pH value of less than 7 within the first hour after birth.

Exclusion Criteria: Newborns having anyone of the following:

- Cyanotic congenital heart disease.
- Rh incompatibility.
- Maternal tobacco addiction.
- Infants of diabetic mothers.
- Preterm.
- H/O of chorioamnionitis.
- Intrauterine growth retardation.

Full-term neonate, Birth weight >2500 g, Apgar score >6 at both one and five minutes, Normal intrapartum foetal heart rate (FHR) pattern, clear amniotic fluid constituted the control group. Cord blood sampling was done soon after birth and the details were entered in a predesigned proforma. This included history regarding antenatal risk factors for perinatal asphyxia such as age of mother, history of pregnancy induced hypertension, anaemia, bleeding, infection and systemic disease. Intrapartum factors like mode of delivery, history of prolonged rupture of membrane, meconium stained amniotic fluid, malpresentation and cord complication (Cord prolapse, Cord around neck, Cord compression) were also entered. Smears were prepared by Leishman stain to determine the nucleated RBC count per 100 WBCs and blood was tested for pH (arterial blood gas studies). Various outcome during hospital stay like time taken to start direct breast-feeding, time taken for good cry/tone/activity/rooting/sucking, time taken to achieve normal neonatal reflexes were recorded in days. Then their final immediate outcome at discharge was categorised into: Neurologically normal (those who had normal tone and posture, were free from seizures, had good cry and activity and normal neonatal reflexes) and neurologically abnormal (those with an abnormal tone and posture or poor cry and activity or any abnormal neonatal reflexes at discharge or death) were also be recorded. Statistical analysis was done using IMSTAT. Discrete variables were analysed by using chi-square test and continuous variable were analysed by using student t test and multiple variable by one way ANOVA test.

RESULTS: The mean NRBC in blood of newborns in cases and controls were 22.63±6.95 (range 9-42 NRBCs/100 WBC) 4.75±2.04 (range of 2-14 NRBCs/100 WBC) respectively. A statistically significant higher NRBC were found in newborns with risk factors (table no. 1) such as antepartum foetal distress i.e. abnormal cardiotocographic changes (p=<0.0001), meconium stained liquor (p=0.0001), cord complication (cord compression, cord around neck, cord prolapse) (p=0.0006) and antepartum haemorrhage (p=0.0114). Mean birth weight in asphyxiated newborns was 2678.65±453 grams and 2807.3±298.3 grams in control which was not statistically significant (p=0.0591).

A highly significant (p=0.0001) negative correlation existed between NRBC count and Apgar score at 1 minute as well as at 5 minutes. After the above observations, a cut-off of 10 NRBC/100 WBCs was taken to differentiate between control (Normal) and case (Asphyxiated) group, and they were grouped as >10 and <10. It was noticed that 58/60 newborns were true positive and 2/60 newborns were false positive with respect to value >10. With cut-off value <10, 4/60 newborn were false negative and 56/60 newborn were true negative, indicating no difference in both categories P=0.0001, Chi-square value = 93.737, degree of freedom=1 was observed which was statistically significant (Table 2).

When degree of validity of NRBCs was further analysed, it had a sensitivity of 96.67%, specificity of 93.33%, positive predictive value of 93.55%, and negative predictive value of 96.55%. NRBC counts were found to be 16.9±4.17 for HIE grade 1, 25.4±3.71 for HIE grade 2, 33.62±4.03 for HIE grade 3 (table no. 3). Among the asphyxiated babies, for correlation between NRBC and various outcomes during hospital stay, we further divided in 3 groups on the basis of number of NRBC/100 WBCs present, 0-10, 11-20 & 21 and more respectively. A statistically significant positive correlation existed between increasing NRBC count and longer time taken to start direct breast-feeding (p=0.0001), recovery of neonatal reflexes (p=0.0001), recovery towards good cry, sucking and activity (p=0.0006) and duration of stay in NICU (p=0.0001) as shown in table no. 4.

For final immediate outcome at discharge, mean nucleated RBC count was compared among the neonates who were neurologically normal (18.45±5.36), neurologically abnormal (24.18±5.53) and who expired (31.70±10.07) after sustaining severe asphyxia, it was seen that the variation was statistically significant (P=0.0001).

	Case (n=60)	Control (n=60)	P value
Age of mother (Mean±SD)	24.2±3.74	24.5±63.4	0.6686
Primigravida Mother	42/60	40/60	0.8444
Period of Gestation in weeks (Mean±SD)	39.53±1.28	39.31±1.08	0.3111
Antepartum Haemorrhage	10/60	1/60	0.0114
Cord Complication*	20/60	4/60	0.0006
Foetal distress (Abnormal CTG† changes)	33/60	7/60	0.0001
Meconium Stained Liquor	30/60	7/60	0.0001
Mean Birth weight in Gram (Mean±SD)	2678.65±453	2807.5±298.3	0.0591

Table 1: Risk Factors For Perinatal Asphyxia in Case & Control Groups

*Cord complication (Cord Prolapse, Cord Compression, Cord around Neck).
†CTG- cardiotocographic changes (Late Deceleration, Absence of Heart Beat to Heart Beat Variability) SD- standard deviation.

NRBC*/100 WBCs Count at Birth	Asphyxia		Total	P value
	Present	Absent		
>10	58	4	62	0.0001
<10	2	56	58	
Total	**60**	**60**	**120**	

Table 2: Diagnostic Validity of Nucleated RBCs in Perinatal Asphyxia

* NRBC- nucleated red blood cell count

Characteristics	NRBC/100WBC	P VALUE
APGAR score at I minute in case group		
>3	18.74±4.41	0.0001
<3	28.87±5.18	
Umbilical cord pH at birth		
7.2 and more	14.16±2.78	0.0001
7.01-7.19	20.05±5.51	
7 and less	27.63±5.73	
Hypoxemic Ischaemic Encephalopathy		
Stage I	16.90±4.17	0.0001
Stage II	25.4 ±3.77	
Stage III	33.62±4.03	
Final immediate outcome at discharge		
Neurological normal	18.45±5.36	0.0001
Neurological abnormal	24.18±5.53	
Death	31.70±10.07	

Table 3: Correlation of NRBC Counts with Apgar Score[2], Cord pH, HIE staging[3] Severity & Final Immediate Outcome at Discharge in Cases (n=60)

Group	NRBC Count at Birth	Time Taken to Start Direct Breast Feeding (Days)		Recovery of Cry/Tone /Activity /Sucking (Days)		Recovery of Neonatal Reflexes (Days)		NICU Stay (Days)	
		mean	SD	mean	SD	mean	SD	mean	SD
1	0 to 10	6	1.41	5	1.41	8	1.41	8	1.41
2	11 to 20	9.55	3.28	8.6	3.18	10.9	3.41	12.05	3.79
3	≥ 21	14.94	5.18	12.97	5.01	16.63	5.21	18.48	5.43
	P value	**0.0001**		**0.0006**		**0.0001**		**0.0001**	

Table 4: Correlation of NRBC Count and Various Outcomes during Hospital Stay in Cases (60)

DISCUSSION: Several authors have evaluated the relationship between various surrogate markers of asphyxia and NRBC count. Spencer et al[4] concluded that NRBC count is a better marker of foetal metabolic acidosis than MSAF, non-reassuring FHR, low Apgar scores, and foetal erythropoietin levels. In our study, statistically significant higher NRBC were found in newborns with risk factors such as antepartum foetal distress i.e. abnormal cardiotocograph, meconium stained liquor, cord complication (Cord compression, Cord around Neck, Cord Prolapse) and antepartum haemorrhage.

These observations were also similar in study done by Saracoglu et al[5] Phelan et al[6] Dasari et al[7] Hanlon-Lundenberg et al.[8] In the present study, nucleated RBC count at birth was found to be 22.63±6.95 in asphyxiated neonates (Cases) and 4.75±2.04/100 WBCs in normal neonates (Control) which is highly significant (p=0.0001). Phelan et al[6] found the mean NRBCs of 3.4±3.0/100 WBCs in healthy neonates and 34.5±68.3/100 WBCs in asphyxiated neonates, and Hassan Boskabadi et al[9] found the NRBC counts to be 3.81±5.06/100 WBCs in healthy infants and 18.63±16.62/100 WBCs in asphyxiated infants.

The present study found a sensitivity of 96.67%, specificity of 93.33%, positive predictive value of 93.55%, and negative predictive value of 96.55% of Nucleated Red Blood Cell (NRBC) count which is similar to Goel M et al[10] study. We also observed significant (p=0.0001) inverse correlation between pH and NRBC count. Cases with cord blood pH of less than 7 had highest NRBC count followed by cord pH 7-7.2 and 7.20 or more. Studies by Thilaganathan et al[11] & Saracoglu et al[5] found a similar significant correlation.

In the present study, we found that the mean NRBC count was highest in neonates who had expired (31.70±10.07) followed by those who were neurologically abnormal at discharge (24.18±5.91) both being significantly higher than those who had favourable outcome eventually (18.45±5.36). This negative correlation was found to be statistically significant (p=0.0001). Ruchi Rai et al[12] found that neonates with abnormal neurological status at discharge had high mean NRBC count (7.9±6.0) as compared to neonates with normal neurological status (4.4±6.6) with a p value of 0.007.

CONCLUSION: With the present study, we strongly advocate that a simple, easily available, cost effective, bedside test, such as NRBC count at the time of delivery is a good marker of perinatal asphyxia and its forthcoming neurological outcome. NRBC count can be a useful part of the paediatrician armamentarium for the evaluation of perinatal asphyxia where facilities of pH sampling are either not available or not cost effective.

REFERENCES

1. World Health Organization. Neonatal and perinatal mortality: country, regional and global estimates, 2004. Geneva: WHO 2006:1-25.
2. Use and abuse of APGAR score. Committee on foetus and newborn, American Academy of Pediatrics and Committee on Obstetric Practice, American College of Obstetrics and Gynecology. Pediatric 1996;98(1):141-142.
3. Hansen AR, Soul. Perinatal asphyxia and hypoxic ischemic encephalopathy. In: John CP. ed. Manual of neonatal care. 7th edn. Philadelphia: Lippincott publication 2011:711-727.
4. Spencer MK, Khong TY, Matthews BL, et al. Haematopoietic indicators of foetal metabolic acidosis. Aust N Z J Obstet Gynaecol 2000;40(3):286-289.
5. Saracoglu F, Sahin I, Eser E, et al. Nucleated red blood cells as a marker in acute and chronic foetal asphyxia. Int J Gynaecol Obstet 2000;71(2):113-118.
6. Phelen JP, Kirkendall C, Korst LM, et al. Nucleated red blood cell and platelet counts in asphyxiated neonates sufficient to result in permanent neurologic impairment. J Matern Foetal Neonatal Med 2007;20(5):377-380.
7. Para D, Jyotsna PG, Ashok BB. Cord blood nucleated red blood cell count – a marker of foetal asphyxia. J Obstetrics and Gynecology India 2008;58(1):45-48.
8. Hanlon-Lundberg KM, Kirby RS, Gandhi S, et al. Nucleated red blood cells in cord blood of singleton term neonates. Am J Obstet Gynecol 1997;176(6):1149-1156.
9. Boskabadi H, Maamouri G, Sadeghian MH, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: a case-control study. Arch Iran Med 2010;13(4):275-281.
10. Goel M, Dwivedi R, Gohiya P, et al. Nucleated red blood cell in cord blood as a marker of perinatal asphyxia. J Clin Neonatol 2013;2(4):179-182.
11. Thilaganathan B, Athanasious S, Ozmen S, et al. Umbilical cord blood erythroblast count as an index of intrauterine hypoxia. Arch Dis Child Foetal Neonatal Ed 1994;70(3):F192-194.
12. Rai R, Tripathi G, Singh DK. Nucleated RBC count as predictor of neurological outcome in perinatal asphyxia. Indian Pediatrics 2014;51(3):231-232.