

TO STUDY THE ROLE OF NUCLEATED RED BLOOD CELLS IN EARLY DIAGNOSIS OF PERINATAL ASPHYXIA, SEVERITY AND OUTCOME**¹Shankey Dudeja, ²Jalaz Jain, ³Gayatri Kumra, ⁴Vibhuti Kumar, ⁵Gourav Siwas, ⁶Drishti Dudeja and ⁷Deepika Jain**¹Junior Resident, Department of Paediatrics, Dr. Baba Saheb Ambedkar Hospital, New Delhi, India.²Senior Resident, Department of Orthopaedics, Satyawdi Raja Harishchandra Hospital, Narela, New Delhi, India.^{3,4}Junior Resident, Department of Anaesthesiology and Critical Care, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India.⁵Junior Resident, Department of Plastic Surgery, Sir Ganga Ram Hospital, Delhi NCR India, India.⁶Undergraduate, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India.⁷Junior Resident, Department of Radiology, Artemis Hospital, Gurgaon, India.***Corresponding Author: Dr. Jalaz Jain**

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ABSTRACT

Perinatal asphyxia accounts for 0.7 to 1.2 million deaths per year.^[1] Perinatal asphyxia is associated with central nervous dysfunction also known as Hypoxic Ischemic Encephalopathy (HIE). Early detection is important in terms of both morbidity as well as mortality. APGAR score is a standardised and simple early tool for the assessment of perinatal asphyxia in newborn immediately after birth. Nucleated red blood cell (NRBC) are produced in fetal bone marrow. NRBC increases in number in fetal hypoxia. In present study, correlation of nucleated red blood cell, cord PH and base excess with APGAR score and HIE grading being done to study severity of perinatal asphyxia and clinical outcomes in neonates.

KEYWORDS: Nucleated RBCs, APGAR, Perinatal asphyxia.**INTRODUCTION**

Worldwide perinatal asphyxia is the leading cause of morbidity and mortality in neonates. World Health organization defines perinatal asphyxia as failure to initiate and sustain breathing at birth.^[2] In addition to central nervous system dysfunction (Hypoxic ischemic encephalopathy) and multiple organ involvement is noted in neonates.^[3]

APGAR score is a standardised and simple tool, consists of five objective signs in newborn. Each sign is evaluated and given a score of 0, 1 and 2. Total scores at 1 and 5 minutes are usually noted. Low APGAR scores at 5 and 10 minutes of age are consistent with an acute peripartum or intrapartum hypoxic event. National Neonatal Perinatal Database has defined moderate asphyxia as slow gasping breathing or APGAR score of 4 – 6 at 1 minute of age and severe asphyxia defined as no breathing or an APGAR score of 0-3 at 1 minute of age.^[4]

Elevated nucleated RBC count can be physiological and can be seen in many conditions- hypoxia, hypoxemia, premature infants, respiratory distress syndrome and preclampsia. In the present study we illustrate that NRBC count and cord pH combination with HIE grade

can prognosticate the risk of complications of asphyxia.^[5]

MATERIAL AND METHODS

This prospective study was conducted in Department of Paediatrics, Dr Baba Saheb Ambedkar Hospital and Medical College, New Delhi. Sample size was 100 (50 each in case group and 50 in control group). Inclusion criteria for cases were term neonates, appropriate for gestation age (AGA), APGAR score < 6 at 1 minute of life, normal breathing not established at 1 minute of life and need for resuscitation with positive pressure ventilation immediately at birth. Newborns fulfilling the inclusion criteria with informed consent of parents were enrolled for the study.

Immediately after birth, 1 ml of cord blood was taken in a heparinised syringe for blood gas analysis from a doubly clamped segment of umbilical cord and another 2 ml cord blood samples were taken in both cases and control group in ethylenediamineacetic acid (EDTA) tube. Along with it a thin blood film was prepared on glass slide, which was dried and stained with leishman's stain. Sample was sent for laboratory analysis.

Sample was processed and analysed in a blinded manner for haemoglobin, total leucocyte count, differential

leucocyte count and haematocrit. Blood smear was examined for detecting number of NRBCs/100 white blood cells (WBCs). Normal healthy controls were kept with mothers and asphyxiated babies (cases) were shifted to Neonatal intensive care unit for resuscitation and management. Grading of severity of asphyxia was done by APGAR score and HIE staging (Sarnat and Sarnat).^[6] Correlation of cord blood NRBC, cord PH and base excess with APGAR score and final outcome was evaluated and analysed statically.

OBSERVATION AND RESULTS

This study was carried out in NICU, Department of Pediatrics, Dr Baba Saheb Ambedkar Hospital and Medical College, New Delhi. The study population consisted of 50 Asphyxiated babies as cases and 50 Non-asphyxiated babies were taken as control. These babies were included in study after taking written and informed consent from parents.

Table 1: Demographic parameters of study groups.

Gender	Case		Control		Total		p value
	N	%	N	%	N	%	
Female	24	48	25	50	49	49	0.841
Male	26	52	25	50	51	51	
Total	50	100	50	100	100	100	

Table shown above depicts gender distribution. Males were more than females in case study group and were equal to females in control study group. Chi square test

application shows that it is statistically insignificant and both groups were comparable in gender distribution. (p = 0.841)

Table 2: Association of Birth weight with Case and Control study groups.

Study Group	Birth Weight	p Value	Statistical Significance
Case	2.91	0.05	Not significant
Control	2.88		

The above table depicts that birth weight among cases and controls. Birth weight in case study group was 2.91

kg and 2.88 kg in control study group and the difference was found to be statistically insignificant (p=0.052).

Table 3- NRBC/100 WBC count among study groups.

Study group	Mean NRBC	SD	P value	Significance
case	17.84	8.74	<0.0001	Statistically significant
control	3.88	2.25		

Above table depicts association of mean NRBC with study groups. In case group mean NRBC count

17.84/100 WBC count while in control group it is 3.88/100 WBC count.

Table 4: Mean Apgar score at 1 and 5 minutes in study group.

Apgar Score	Case	Control	p value	Significance
Apgar at 1 min	2.98±0.96	7.38±0.57	0.0001	Statistically significant
Apgar at 5 min	4.32±1.04	8.72±0.67	0.0001	Statistically significant

The above table depicts comparison of Apgar score at 1 and 5 minutes in case and control study groups. In comparison with the controls, the cases had a

significantly lower Apgar score during the first minute and five minutes post partum (p<0.001).

Table 5: Comparison of Cord blood pH and Base excess in study groups.

	Case	Control	p Value	Significance
Cord pH	7.09	7.41	<0.0001	Significant
Base excess	-11.45	1.8	<0.0001	Significant

Above table depicts association of cord blood pH and base excess with mean NRBC count. Cord blood pH in case group is 7.09 while in control group is 7.41 and the difference is statistically significant (P<0.00001). Cord blood base excess in case group is -11.45 mmol/L while in control group is 1.8 mmol/L (p=<0.0001).

Table 6: HIE Staging in case and control study groups.

HIE Staging	Case	Control	Total	p Value
Stage 1	15(30 %)	0(0%)	15(30 %)	<0.0001
Stage 2	18(36%)	0(0%)	18(36%)	
Stage 3	8 (16%)	0(0%)	8 (16%)	
No HIE	9 (18%)	50(100%)	59(59%)	
Total	50(100%)	50(100%)	100(100%)	

The above table reveals the association of HIE staging with case and control study groups. In the case study group, 36% of cases were from HIE Stage 2 followed by 30% cases attributed to HIE Stage 1 followed by 16%

cases in HIE Stage 3 and 18% of cases were found to have no HIE. In control study group all controls belong to non asphyxiated group.

Table 7 – Relationship of severity of asphyxia and NRBC/100 WBC count.

	Moderate asphyxia	Severe asphyxia	p value
Sample size	18	32	<0.0001
	NRBC/100 WBC		
Mean \pm SD	9.89 \pm 3.51	22.31 \pm 7.52	
Median	9.5	21	

This table depicts relationship between severity of asphyxia and mean NRBC count in asphyxiated babies. In Moderate asphyxiated babies mean NRBC/100 WBC count seen was 9.89 \pm 3.51 while in severe asphyxiated

babies mean NRBC/100 WBC count seen was 22.31 \pm 7.52 which proves NRBC count increases with severity of asphyxia(p=<0.0001).

Table 8: Association of Mean NRBC with HIE Stage in asphyxiated babies.

Birth Asphyxia	HIE Staging	Total	Mean NRBC	p value
No HIE		09	7.33	<0.001
HIE	HIE 1	15	14.7	
	HIE 2	18	21.89	
	HIE 3	8	26.5	

The above table depicts relationship of HIE Staging with mean NRBC/100 WBC count in asphyxiated babies. Mean NRBC/100 WBC was highest among cases with HIE III (26.5/100 WBC) followed by HIE II cases (21.89/100 WBC) and HIE I cases (14.7/100WBC).

NRBC count was minimum in cases with no HIE (7.33/100 WBC). This difference in NRBC count in relation to HIE stage among asphyxiated babies was found to be statistically significant (p<0.001).

Table 9: Association of base excess with final outcome in asphyxiated babies.

		Final outcome		Total	p value
		Death	Discharge		
Base Excess	<-10	48.00%	52.00%	100.00%	<0.0001
	0 to -10	0.00%	100.00%	100.00%	
	>0	0.00%	100.00%	100.00%	
Total		24.00%	76.00%	100.00%	

The above table depicts association of base excess with final outcome in asphyxiated babies. We found out more number of deaths in negative base excess; 48% babies

expired in base excess <-10, no death in base excess range 0 to -10 and <0 base excess respectively. This is statistically significant, as p value is <0.0001.

Table 10: Association of Cord blood pH with final outcome in asphyxiated babies.

		Final outcome			p value
		Death	Discharge	Total	
Cord pH	\geq 7.10	0(0.00%)	14(100.00%)	14(100.00%)	<0.0001
	7-7.10	3(12.00%)	22(88.00%)	25(100.00%)	
	<7	9(81.82%)	2(18.18%)	11(100.00%)	
Total		12(24.00%)	38(76.00%)	50(100.00%)	

Above table depicts association of Cord pH with final outcome in asphyxiated babies. 81.82% babies expired in cord pH < 7 and rest discharged. 12% babies expired in pH range 7 to 7.10 and 88% babies discharged while no

baby expired in pH >7.10 and 100% of babies were discharged. The mean cord blood pH was low in cases having unfavorable final outcome.

Table 11: Correlation of APGAR score at 1 and 5 minutes with NRBC/100 WBC Count in asphyxiated babies.

APGAR score	N	Spearman correlation coefficient	p value
At 1 minute	50	-0.866	<0.0001
At 5 minute	50	-0.875	<0.0001

Correlation coefficient between NRBC/100 WBC count, APGAR at 1 minute, APGAR at 5 minutes was analysed for the case group. There was significant negative correlation of NRBC count with APGAR at 1 minute,

APGAR at 5 minutes and with coefficient values of -0.866, -0.875 respectively with significant p value (<0.001)

Table 12: Correlation between HIE Staging and Mean NRBC in asphyxiated babies.

	N	Spearman correlation coefficient	P value
HIE Staging	50	0.950	<0.0001

Correlation coefficient between NRBC/100 WBC count and HIE staging was analysed for the asphyxiated subjects. There was significant positive correlation of

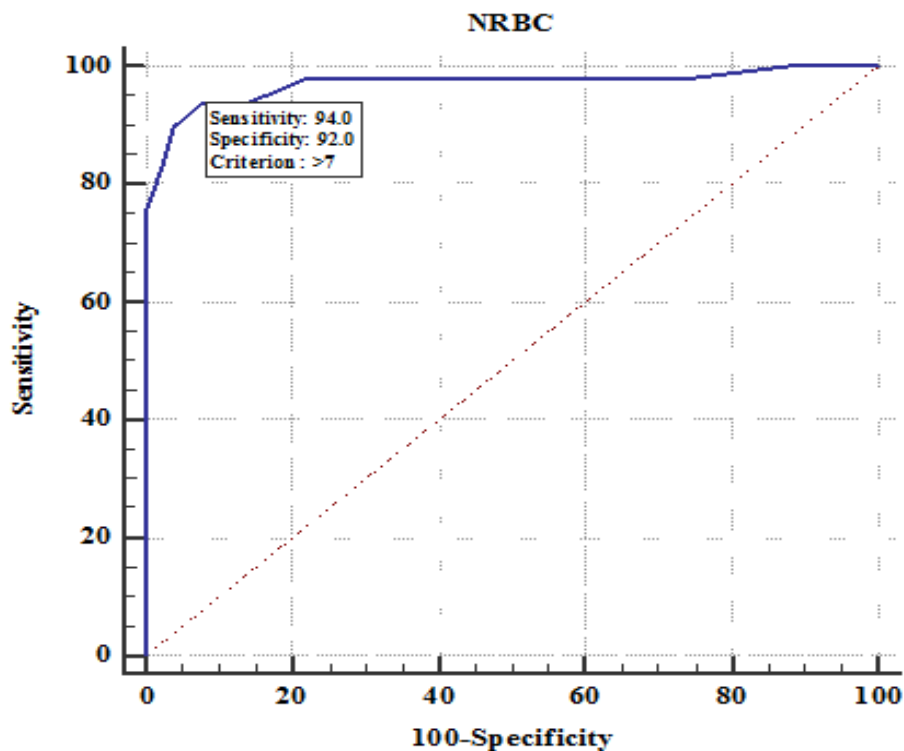
NRBC/100 WBC count with HIE staging and with coefficient value of 0.950 respectively with significant p value (<0.001).

Table 13:- Sensitivity, specificity, positive predictive value and negative predictive value of NRBC count in asphyxiated babies.

	AUC	SE	95% CI	p value	Cut off	Sensitivity	Specificity	PPV	NPV
NRBC/100 WBC	0.971	0.0175	0.917 To 0.994	<0.0001	>7	94	92	92.2	93.9

ROC curve was plotted for the cut off value of NRBC count (NRBC/100 WBC) of 7 for predicting the asphyxia with umbilical cord pH value of <7.20. The sensitivity

and specificity were 94% and 92% respectively. The positive predictive value (PPV) was 92.2% and negative predictive value (NPV) was 93.9%.



DISCUSSION

Purpose of this study was to evaluate role of NRBC/100 WBC in early diagnosis of birth asphyxia, severity and its outcome in term neonates. Birth weight in case study group was 2.91 kg and 2.88 kg in control study group). Similarly, in the study done by Mohanty et al (2014)^[7], birth weight in case study group was 2.86 kg and 2.77 kg in control study group and all these clinical variables were comparable in both the groups without significant difference ($p > 0.05$).

In comparison with the controls, the cases had a significantly lower Apgar score during the first minute and five minutes post partum ($p < 0.001$) which is comparable to findings of study done by Ganta SJ et al (2017).^[8] Similarly, study done by Meena et al (2016)^[9] also depicted that the mean APGAR at 1 and 5 minutes in the case group was lower (3.76 ± 1.04 and 5.82 ± 1.88) in comparison to control group (8.20 ± 0.64 and 9.28 ± 0.45) with p value < 0.0001 .

In case group, Mean NRBC/100 WBC count is 17.84 while in control group it was 3.88/100 WBC count and difference was found to be statistically significant ($P = < 0.0001$) which is comparable to study by Meena et al (2016)^[9] in which mean NRBC (NRBC/100 WBC) count was 17.82 in case group and 1.42 in control group ($p < 0.001$). Similarly, in the study done by Colaco et al (2014)^[10] had the mean NRBC/100 WBC of 17.43 ± 19.86 in cases and 2.97 ± 4.79 in controls.

Cord blood pH in case group was 7.09 while in control group was 7.41 and the difference was statistically significant ($p < 0.00001$). Cord blood base excess in case group is -11.45 mmol/L while in control group was 1.8 mmol/L ($p = < 0.0001$). In lines with this data, Mannan MA (2019)^[11] and coworkers showed that worsening umbilical cord pH and base excess correlated with increasing severity of HIE and also with mortality.

In present study, NRBC/100 WBC in asphyxiated subjects were highest in 11-30 range (70%) followed by 1-10 NRBC range (24%) and 6% of NRBC in > 30 NRBC range. Most of the controls (96%) had NRBC/100 WBC count in range of 1-10, while 4% controls did not show any NRBC/100 WBC. No control had NRBC count in range of 11-30. This difference in NRBC/100 WBC range among case and control groups was found to be statistically significant ($p < 0.001$) which is comparable to study done by Ganta SJ et al (2017).^[8]

Subjects were divided according to Sarnat and Sarnat^[6] scoring system into three stages. In case study group, 36% of cases were from HIE Stage 2 followed by 30% cases attributed to HIE Stage 1 followed by 16% cases in HIE Stage 3 and 18% of cases were found no HIE. In control study group all controls belong to no HIE Group ($p < 0.0001$). In study conducted by Elfarargy et al (2018)^[12] showed, 30% were classified as grade I HIE, 40% as grade II HIE and 30% as grade III HIE. Mean

NRBC was highest among cases with HIE III (26.5/100 WBC) followed by HIE II cases (21.89/100 WBC) and HIE I cases (14.7/100 WBC). NRBC/100 WBC count was minimum in cases with no HIE (7.33/100 WBC). This difference in NRBC count in relation to HIE stage among asphyxiated babies was found to be statistically significant ($p < 0.001$).

In moderate asphyxiated babies 9.89 mean NRBC/100 WBC count seen while in severely asphyxiated babies 22.31 mean NRBC/100 WBC count was seen which proves NRBC/100 WBC count increases with severity of asphyxia ($p = < 0.0001$).

There was significant negative correlation of NRBC/100 WBC count with APGAR at 1 minute, APGAR at 5 minutes and with coefficient values of -0.866 , -0.875 respectively with significant p value < 0.001 . Similarly, in the study done by Colaco et al (2014),^[10] negative correlation was seen between mean NRBC/100 WBC count and APGAR score at 1 and 5 minutes. There was significant negative correlation of NRBC count with Cord pH, Base Excess and with coefficient values of -0.888 , -0.920 respectively with significant p value (< 0.001). Similarly, in the study done by Lundberg et al (1999),^[13] negative correlation was seen between mean NRBC/100 WBC count and cord pH, base excess.

ROC curve plotted for the cut off value of NRBC/100 WBC count for predicting the asphyxia with umbilical cord pH value of < 7.20 . The sensitivity and specificity were 94% and 92% respectively. The positive predictive value (PPV) was 92.2% and negative predictive value (NPV) was 93.9%. In study done by Shrivastava et al (2018),^[14] ROC curve was plotted with NRBCs with cutoff ≥ 10 has sensitivity of 88.75%, specificity of 100%, positive predictive value of 100% and negative predictive value of 89.89%.

CONCLUSION

In conclusion, present results showed that the NRBC/100 WBC count in cord blood at birth can be considered as a good marker of perinatal asphyxia. A simple, cheap, rapid and non-invasive test of cord blood NRBCs count provides valuable information about the well-being of the new-born at birth and it correlates well with APGAR score and cord blood pH. The NRBC/100 WBC count can hence be used as a simple tool to predict the severity and immediate outcome of birth asphyxia with sensitivity of 94%, specificity of 92%, PPV of 92.2% and NPV of 93.9%.

DECLARATION

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CONFLICTS OF INTEREST- None declared.

ETHICAL APPROVAL- Study was approved by Institutional review board and Ethical committee.

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