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Original Research Article

A STUDY OF NUCLEATED RED BLOOD CELL COUNT IN VENOUS BLOOD AS AN EARLY PREDICTOR OF SHORT-TERM OUTCOME IN PERINATAL ASPHYXIA

K. Kamatchi[1], U. Sasireka[2], K. Nurpavi[3], V. Gnanasekaran[4]

[1]Assistant Professor, Department of Pediatrics, Government Karur Medical College Hospital, Tamilnadu, Inida.
[2]Assistant Professor, Department of Pediatrics, Government Karur Medical College Hospital, Tamilnadu, Inida.
[3]Postgraduate, Department of Pediatrics, Government Thoothukudi Medical College Hospital, Tamilnadu, Inida.
[4]Assistant Professor, Department of Pediatrics, Government Thoothukudi Medical College Hospital, Tamilnadu, Inida.

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Corresponding Author:
Dr. V. Gnanasekaran,
Email: sasignans@gmail.com.

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Abstract

Background: Perinatal asphyxia is a major cause of neonatal mortality. It leads to organ dysfunction, including hypoxic-ischaemic encephalopathy (HIE), which can cause neurological complications. Several indicators predict the severity of perinatal asphyxia, including APGAR scores and neoplastic red blood cells. This study aimed to analyse the relationship between nucleated RBC counts and short-term outcomes in terms of neonatal morbidity and mortality in perinatal asphyxia. **Material and Methods:** This prospective observational cohort study was conducted on 100 intramural term asphyxiated neonates at the Government Thoothukudi Medical College and Hospital for 18 months from January 2019 to June 2020. Details of maternal determinants, such as age, obstetric code, maternal comorbidities, blood group, drugs taken during pregnancy, and details of labour and delivery, were collected and recorded in a case format. **Results:** The differences were statistically significant in the mode of delivery, foetal distress, PPV, and mean cord blood pH($p<0.05$). NRBC levels were found to differ significantly across mild, moderate, and severe HIE groups, as well as among individuals with different seizure occurrences. The use of primary and secondary anticonvulsants was correlated with elevated NRBC levels, demonstrating a statistically significant association. Additionally, cord blood pH varied significantly among the HIE severity groups. Higher NRBC levels were observed in patients who experienced mortality or displayed an abnormal neurological status, and these associations were also statistically significant. **Conclusion:** The nucleated red blood cell (NRBC) count is crucial for diagnosing and predicting perinatal asphyxia, especially in severe stages of HIE, especially in countries such as India where cord blood collection is challenging.

INTRODUCTION

Perinatal asphyxia is one of the most significant causes of neonatal mortality. Perinatal asphyxia accounts for 23% of 4 million neonatal deaths every year globally and contributes to 20% of neonatal deaths in India, as per the National Neonatal-Perinatal database.[1] Perinatal asphyxia is defined as "Failure to initiate and sustain breathing at birth" by the World Health Organisation (WHO).[2] As a result of asphyxia, various organ dysfunction will occur due to lack of perfusion and oxygen. Among the various organ dysfunctions, Hypoxic ischemic encephalopathy (HIE) is one of the most common comorbidities leading to neurological sequelae.[3,4] So, early prediction of hypoxic-ischemic encephalopathy is important for further interventions. Hypoxic-ischaemic encephalopathy (HIE) is described as stages 1, 2, and 3 based on the severity after clinical examination. HIE should meet the following criteria for acute neurological injury: Persistence of an Apgar score of less than 3 for more than 5 min. Profound metabolic or mixed acidemia (pH<7) in umbilical cord blood and base deficit >10meq/l. Neurological dysfunction is characterised by seizures, motor abnormalities, encephalopathy, brainstem and cranial nerve abnormalities, and increased intracranial pressure

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(ICP). Evidence of multiorgan dysfunction (kidneys, heart, lungs, liver and intestines).[5,6]

Most HIE cases develop neurological morbidity in the form of seizures, developmental delay, cerebral palsy, and mental retardation on short-term and long-term follow-up.[7] Clinical examination can confirm the severity of hypoxic-ischaemic encephalopathy within 24 hours of birth.[8] Many indicators are available to predict the severity of perinatal asphyxia at the time of birth, such as APGAR score at 1 min, cord blood pH, and base deficit. In addition, nucleated RBCs in the blood are emerging as a newer indicator of the severity of Birth Asphyxia.[9] Considering the haematopoietic response to hypoxia in utero, the increased NRBC count has been investigated as a marker of asphyxia in various studies.

Previous studies have assessed the ability of nucleated blood cells as a marker of perinatal asphyxia. However, only a few studies have assessed the severity of hypoxic-ischaemic encephalopathy.[10] The nucleated RBCs level per 100 white blood cells (WBCs) correlates well with acute and antepartum asphyxia. It can be used as a reliable index of perinatal asphyxia and short-term neonatal outcomes.[11] Most of the studies used cord blood to report NRBC count. In developing countries such as India, collecting cord blood is difficult, as most deliveries occur in peripheral health centres and homes. Therefore, the present study was undertaken to evaluate venous blood NRBC counts as a marker of perinatal asphyxia in conjunction with other clinical markers and its ability to predict the severity of perinatal asphyxia and its immediate outcome in term newborns.

Aim

This study aimed to analyse the relationship between nucleated RBC counts and short-term outcomes in terms of neonatal morbidity and mortality in perinatal asphyxia.

MATERIALS AND METHODS

This prospective observational cohort study was conducted on 100 intramural term asphyxiated neonates at the Government Thoothukudi Medical College and Hospital for 18 months from January 2019 to June 2020. The study was initiated after obtaining proper approval from the Ethical Committee, and informed consent was obtained from the parents.

Inclusion Criteria

Allterm intramuralasphyxiated newborn babies, as per the WHO 2006 guidelines, "Failure to Initiate and Sustain Breathing at Birth", and willingness for consent were included.

Exclusion Criteria

Extramural asphyxiated babies, intrauterine growth-restricted babies, preterm babies with a gestational age of <37 weeks, maternal uncontrolled PIH or GDM, babies with congenital malformations, babies with Rh and ABO incompatibility and other haemolytic disorders, babies born to mothers with a PROM history of more than 18 hours, mothers on drugs which alter their haematological profile, such as beta-blockers, ACE inhibitors, and thiazides, and those not willing to consent were excluded.

100-term intramural neonates with asphyxia were enrolled after excluding babies based on the exclusion criteria. They were further classified into moderate and severe asphyxia groups based on Apgar scores at 1 min of life. Babies with low Apgar scores at 1 min were followed up with their 5-minute Apgar scores. After the initial resuscitative measures, once the neonates were stabilised, 1 ml of cord blood was collected in a pre-heparinised syringe for pH analysis and transferred to the NICU for post-resuscitation care. Within 6 h of life, 2 ml of venous blood was collected in an EDTA tube for peripheral smears.

Details of maternal determinants, such as age, obstetric code, maternal comorbidities, blood group, drugs taken during pregnancy, and details of labour and delivery, were collected and recorded in a case format. Baby determinants such as date of birth, sex, gestational age, birth weight, Apgar score at 1 and 5 min, resuscitation details (according to AAP guidelines, 2015), and who required PPV for more than 1 min were recorded. Using Sarnat and Sarnat staging 41, admitted babies were neurologically assessed and further classified into stages 1, 2, and 3 HIE. The type of respiratory support required, presence of seizures, need for primary and secondary anticonvulsants, duration of hospital stay, and short-term outcome, either in death or discharge (if discharged, normal, or abnormal) were noted.

A modified New Ballard score was used to assess gestational age. Cord blood pH analysis was performed using an analyser as soon asit was obtained. Then, two ml of venous blood was collected within 6 h of birth in an ethylenediaminetetraacetic acid (EDTA) tube and transferred to the pathology department. The samples were refrigerated if the processing wasdelayed. The blood smears were fixed with ethanol andstained with Leishman's stain, and a manual differential count was performed to count NRBCs and reported as NRBCs / 100 WBCs by a blinded pathologist.

Statistical Analysis

Means and standard deviations were calculated for continuous variables, and percentages were calculated for categorical variables, such as sex and mode of delivery. The Student's t-test was used to compare the statistical significance between the means of the two groups. The chi-squared test was used for categorical variables. The correlation coefficient and area under the curve were used to assess the strength of predicting the severity of asphyxia based on nucleated RBC count. Statistical significance was set at $p < 0.05$.

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RESULTS

Of these, 74 patients had moderate asphyxia (APGAR score of 4-6 at 1 min of life), and 26 patients had severe asphyxia (APGAR score of 0-3 at one minute of life). Both groups were found to have similar mean gestational age and birth weight and were statistically significant. Both groups were similar imparity distribution, and the difference was not statistically significant (p>0.05). Forty (54.1%) and 23 (88.5%) patients were males in the moderate asphyxia and severe asphyxia groups, respectively. Male patients were found to have more severe asphyxia than female patients, and the association was statistically significant (p < 0.05). Of the 100 cases, 63% were delivered through normal vaginal delivery, 6% through assisted vaginal delivery, and 31% through LSCS.

One study found that 1.4% of babies with moderate asphyxia underwent assisted vaginal delivery, with 19.2% experiencing severe asphyxia. Foetal distress was the highest in babies with severe asphyxia, with 40.5% requiring PPV > 1 min. There was a significant difference in the mode of delivery, foetal distress, and PPV between the groups. The group with severe asphyxia had a relatively lower pH than the group with moderate asphyxia. The difference was statistically significant for nucleated RBCs and mean cord blood pH(p<0.05). [Table 1]

NRBC levels were found to differ significantly across mild, moderate, and severe HIE groups as well as among individuals with different seizure occurrences. The use of primary and secondary anticonvulsants was correlated with elevated NRBC levels, demonstrating a statistically significant association. Additionally, cord blood pH varied significantly among the HIE severity groups. Higher NRBC levels were observed in patients who experienced mortality or displayed an abnormal neurological status, and these associations were also statistically significant. [Table 2]

Severe HIE had a higher proportion of nucleated RBCs than moderate and mild HIE. The difference in each stage was statistically significant compared with the other stages. Mean NRBC levels were higher in the seizure group than in the control group. The severity of HIE increased when the cord blood pH decreased, and this was found to be statistically significant (p<0.05). [Table 3]

The area under the curve was 0.897, which was statistically significant. The cutoff point of NRBC was 15.5, meaning<15.5, mild or moderate HIE, and severe HIE status of > 15.5. When the cutoff point for nucleated RBCs was fixed at 15.5, the sensitivity and specificity were 92.9% and 79.1%, respectively. The area under the curve was 0.673, which was statistically significant. The cutoff point was 10.5, meaning<10.5, mild HIE status, and more than 10.5 was moderate or severe HIE status, respectively. When the cutoff for nucleated RBCs was 10.5, the sensitivity was 80.8% and the specificity was 66.2%. For mild HIE, the cutoff point was <10.5, the sensitivity was 80%, and the specificity was 67%. So, the cutoff of NRBC for predicting mild HIE was <10.5, severe HIE was>15.5 and moderate HIE status was between > 10.5 and 15.5 (Table 4, Figures 1 and 2).

Among the study participants with abnormal neurological status, two (20%) had severe HIE staging, and among those with normal neurological status, none had severe HIE staging. The association ofbabies with increased NRBCs is at risk of severe HIE with abnormal neurological status, which is statistically significant (p<0.05).

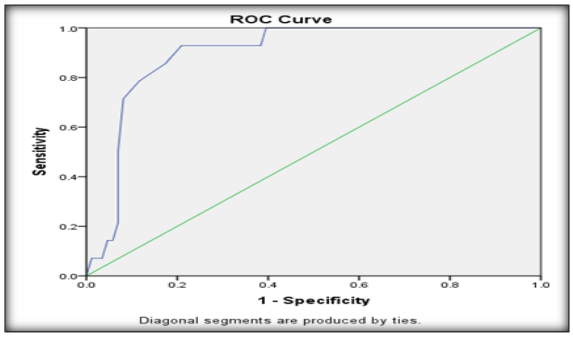

Figure 1: ROC curve for diagnosing severe HIE

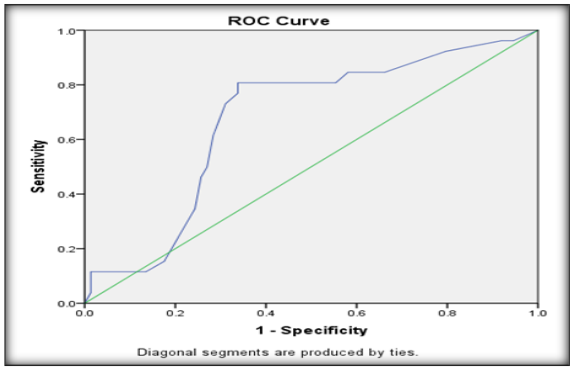

Figure 2: ROC curve for diagnosing moderate HIE

Both Nucleated RBCs and Cord blood pH were negatively correlated with each other. With each unit increase in nucleated RBCs, there was an approximately a 0.4-unit decrease in cord blood pH. (Table 5 and Figure 3)

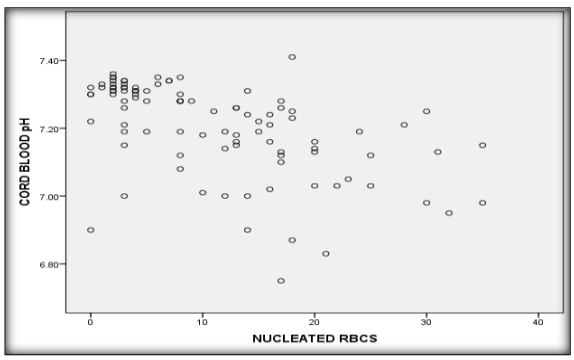

Figure 3: Relationship between Nucleated RBCs and Cord Blood pH

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Table 1: Association of various findings between moderate and severe asphyxia

		Group	
		Moderate asphyxia	**Severe asphyxia**
Gestational age		38.54 ± 1.22	38.3 ± 1.04
Birth weight		2.88 ± 0.31	2.88 ± 0.34
Parity	Primi para	54 (73%)	16 (61.5%)
	Multi para	20 (27%)	10 (38.5%)
Sex of baby	Male	40 (54.1%)	23 (88.5%)
	Female	34 (45.9%)	3 (11.5%)
Mode of delivery	Normal Vaginal delivery	48 (64.9%)	15 (57.7%)
	Assisted vaginal delivery	1 (1.4%)	5 (19.2%)
	LSCS	25 (33.8%)	6 (23.1%)
Foetal distress	Present	43 (58.1%)	22 (84.6%)
	Absent	31 (41.9%)	4 (15.4%)
PPV > 1 min	Yes	30 (40.5%)	26 (100%)
	No	44 (59.5%)	0
Nucleated RBCs		8.07 ± 6.68	19.38 ± 9.12
Mean Cord blood pH		7.25 ± 0.09	7.05 ± 0.12

Table 2: Association between Nucleated RBCs and various findings

		Nucleated RBCs.		Significance
		Mean	**Standard Deviation**	
HIE staging	Mild	6.92	6.624	<0.05
	Moderate	14.81	8.542	
	Severe	21.5	6.061	
Seizures	< 12 hrs	13	2.646	<0.05
	Yes	17.42	8.673	
	No	7.13	6.779	
Primary anticonvulsants	Yes	17.08	8.43	<0.05
	No	7.13	6.77	
Secondary anticonvulsants	Yes	19.11	8.19	<0.05
	No	9.23	8.03	
Mean cord blood pH	Mild	7.2825	0.06519	<0.05
	Moderate	7.1292	0.11496	
	Severe	7.0121	0.12711	
Outcome	Death	24.07	5.92	<0.05
	Discharged	8.71	7.16	
Neurological status	Abnormal	13.9	8.98	<0.05
	Normal	8.01	6.65	

Table 3: The difference in mean nucleated RBCs between HIE staging, seizure, and cord blood pH levels

(I) HIE stage		**Mean Difference (I-J)**	**Sig.**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Mild	Moderate	-7.891*	0	-12.04	-3.75
	Severe	-14.583*	0	-19.82	-9.34
Moderate	Severe	-6.692*	0.021	-12.54	-0.84
(I) Seizures		**Mean Difference (I-J)**	**Sig.**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
< 12 hrs	Yes	-4.417	0.617	-15.57	6.73
	No	5.869	0.416	-5.1	16.84
Yes	No	10.286	0	6.39	14.19
(I) HIE stage and cord blood pH levels		**Mean Difference (I-J)**	**Sig.**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Mild	Moderate	.15327*	0	0.1006	0.206
	Severe	.27036*	0	0.2037	0.337
Moderate	Severe	.11709*	0.001	0.0427	0.1915

Table 4: The area under the curve for diagnosing severe and moderate HIE

HIE	**Area**	**Asymptotic Sig**	**Asymptotic 95% Confidence Interval**	
			Lower Bound	**Upper Bound**
Severe	0.897	0	0.828	0.966
Moderate	0.673	0.009	0.556	0.791

Table 5: Correlation between NRBCs and Cord blood pH

NUCLEATED RBCS	**CORD BLOOD pH**
Pearson Correlation	-.416**
Sig. (2-tailed)	.000
N	85

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DISCUSSION

This study was conducted for 18 months in the NICU of Government Thoothukudi Medical College and Hospital, Thoothukudi, to establish the relationship between the nucleated red blood cell (NRBC) count in peripheral venous blood {which was collected within 6 hours of birth} and perinatal asphyxia, and to assess NRBC count as an indicator or predictor of perinatal asphyxia severity and severity of hypoxic-ischaemic encephalopathy in 100 asphyxiated neonates who developed HIE.

There were no significant differences in gestational age, parity, birth weight of the baby, or perinatal asphyxia ($p>0.05$). The severity of asphyxia was statistically significant in male babies compared to female babies ($p <0.05$). Babies born through assisted vaginal delivery had severe asphyxia (19.2%) compared with those born through LSCS or normal vaginal delivery ($p<0.05$). Foetal distress was significantly correlated with severe asphyxia ($p<0.05$). Babies who were severely asphyxiated required positive pressure ventilation for >1 min compared to those with moderate asphyxia, and this was statistically significant ($p <0.05$).

The Apgar score at 1 min of life and NRBC count were also comparable between the study groups and were found to be statistically significant ($p<0.05$). The study by Perrone et al. showed that 1 and 5 min of APGAR were reciprocally related to the NRBC count.[12] Likewise, in our study, newborns with low Apgar scores revealed a high NRBC count. The mean number of nucleated red blood cells in the moderate asphyxia group was 8.07 ± 6.68; in the severe asphyxia group, it was 19.38 ± 9.12 in our study, which wasstatistically significant ($p<0.05$). NRBC count was higher in the asphyxiated babies than in non-asphyxiated babies in Phelan et al. and Tungalag et al. studies.[13,14]

According to the studies of Tungalag et al. and Saracoglu et al., the association between NRBC count and the severity of HIE was found to be statistically significant.[14,15] We also assessed this relationship in our study and found a significant correlation with a p-value < 0.05. In our study, the mean NRBC levels among mild, moderate, and severe HIE were 6.92 ± 6.62, 14.81 ± 8.54 and 21.5 ± 6.06, respectively, comparable to other studies, including Boskabadi et al., who reported the lowest percentage (6.92%) of mild HIE, while Shivaprakash et al. and Ferns et al. showed higher percentages (11.94% and 10.43%, respectively).[16-18] All three studies showed an increasing trend in the percentage of moderate HIE (Boskabadi et al.: 14.81%, Shivaprakash et al.: 21.08%, Ferns et al.: 18.63%).Fern et al. reported the highest percentage of severe HIE (30.83%), while Boskabadi et al. and Shivaprakash et al. reported lower percentages (21.5% and 29.18%, respectively).[16-18]

Our study found a positive correlation between NRBC count and severity of HIE using regression analysis, signifying thatincreased NRBC count can predict the severity of hypoxic-ischaemic encephalopathy. Goel et al. also evaluated this association and established that the higher the HIE staging, the higher the mean NRBC/100 WBC count.11 Using the Receiver Operator Characteristic curve (ROC), the cut-off points of NRBC for HIE severity were anticipated in our study. The cut-off point for mild HIE was <10.5 with a sensitivity and specificity of 80% and 67%, respectively; for moderate HIE, the cut-off was between 10.5 and 15.5 with a sensitivity and specificity of 80.8% and specificity of 70.2%, respectively; and for severe HIE, the cut-off was >15.5, with a sensitivity and specificity of 92.8% and 79.1%, respectively. The best cut-off value of NRBC count for predicting HIE by Blackwell et al. was 15 NRBCs per 100 WBCs, with a sensitivity of 100% and specificity of 73%.

We found that the mean NRBC count was higher in the group with seizures (17.42 ± 8.67) than in those without seizures (7.13 ± 6.77), and this association was confirmed to be statistically significant with a post-hoc test. The need for anticonvulsants also increased with increasing NRBC counts. Cord blood pH was found to be inversely related to HIE severity in our study using a post-hoc test. The mean cord blood pH for mild, moderate, and severe HIE was 7.28 ± 0.65, 7.12 ± 0.11 and 7.01 ± 0.12, respectively. HIE severity increased with a statistically significant decrease in cord blood pH.

In our study, the NRBC count and cord blood pH were negatively correlated, which was statistically significant ($p<0.05$). With each unit increase in NRBC count, there was an approximately a 0.4-unit decrease in cord blood pH. Mortality was approximately 15%, and the mean NRBC count was significantly higher in the mortality group (24.07 ± 5.92) than in the discharged group (8.71 ± 7.16) ($p < 0.05$). The mean NRBCs were higher in those with abnormal neurological status on discharge (13.9 ± 8.98) than in the study participants with normal neurological status on discharge (8.01 ± 6.65), and it was also found to be statistically significant with a p value < 0.05.

CONCLUSION

The nucleated red blood cell (NRBC) count is an authentic test to differentiate between moderate and severe asphyxia based on the APGAR score at 1 min of life. An increased NRBC countcorrelates positively with the three stages (mild, moderate, and severe) of HIE, especially in the severe stage based on the Sarnat and Sarnat classification. It also predicts the short-term outcomes in terms of mortality. It isn't easy to collect cord blood in countries like India, where most deliveries occur in peripheral centres and at home. Therefore, we inferred that the NRBC count in peripheral venous

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blood collected within 6 h of life plays a role in diagnosing and predicting perinatal asphyxia.

Limitations

As this study involved 100 asphyxiated neonates divided into two groups based on their Apgar score at 1 min of life, the sample size was not equally distributed between the two groups. This study cannot be generalised to the neonatal population because it only involved term newborns. Although we excluded confounding factors such as pregnancy-induced hypertension, gestational diabetes, sepsis, and intrauterine growth restriction from our study, other variables could have affected the level of NRBCs in the circulation. As this was a short-term follow-up study, we followed the babies only until discharge. Therefore, the association between NRBC count and long-term neurological outcomes could not be estimated.

Recommendations

NRBC count is a highly reliable and emerging marker of perinatal asphyxia to assess its severity and predict the pre-discharge outcome of asphyxia in hospitals. It is a simple and inexpensive tool for evaluating asphyxia, especially in resource-poor settings where cord blood gas analysis-like facilities are not available.

Financial interest

Conflicts of interest

Acknowledgement

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