Original Research Article

Cord blood nucleated red blood cell count as a predictor of long term sequelae in cases of perinatal asphyxia: a one-year follow-up study

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ABSTRACT

Background: Nucleated red blood cells (NRBCs) can be easily found in cord blood and its >20/100 WBCs has been distinguished as a marker of perinatal asphyxia at birth. Authors conducted this study to find out if there exists a relation between NRBCs at birth and its effects on long term neurological outcome in patients of perinatal asphyxia.
Methods: This was a prospective longitudinal study conducted in the Department of Pediatrics in collaboration with Department of Obstetrics and Gynaecology, G.S.V.M. Medical College, Kanpur from December 2014 to September 2016.
Results: On assessing the long term neurodevelopmental outcome in newborns with HIE at birth, Authors found that cord blood NRBCs had no direct influence on the final neurodevelopmental outcome at 1 year of life as did HIE staging.
Conclusions: Authors propose that cord blood NRBC counts of > 20/100 WBCs is a good predictor of asphyxia at birth but is definitely not an indicator of forth coming developmental delay.

Keywords: Long term neurodevelopmental outcome, Nucleated RBCs, Perinatal asphyxia

INTRODUCTION

Perinatal asphyxia is a major cause of neonatal mortality and long term neurological disability amongst survivors. It affects about 2-10% births and kills about 19% of over 5 million newborns every year-round the globe.[1,2] Indian data of neonatal deaths also indicates 20% contribution of perinatal asphyxia.[3]

Nucleated red blood cells (NRBCs) can be easily found in neonatal blood and its count in neonatal umbilical venous blood has been distinguished as a marker of perinatal asphyxia.[4] The NRBCs per hundred white blood cells are seldom more than ten in normal newborns.[5] It has been proposed that perinatal asphyxia caused an increased production of erythropoietin leading to increase

in number of nucleated RBCs. NRBCs can be easily detected through simple diagnostic tests even at primary health centre level and thus helps in diagnosing the condition.

Various studies have been conducted to find out NRBCs as the predictor of severity of perinatal asphyxia at birth. Authors conducted this study to find out if there exists a relation between NRBC counts at birth and its effects on long term neurological outcome in patients of perinatal asphyxia.

METHODS

This was a prospective longitudinal study conducted in the Department of Pediatrics in collaboration with

Department of Obstetrics and Gynaecology, G.S.V.M, Medical College, Kanpur from December 2014 to September 2016. The study was approved by ethical committee of G.S.V.M, Medical College, Kanpur. Sixty out of the total neonates delivered in present hospital from December 2014 to September 2015 and meeting the inclusion criteria for perinatal asphyxia were selected for study and sixty neonates with normal perinatal outcome were selected as controls. An informed written consent from parents was taken and children were further followed-up for one year till September 2016.

Inclusion criteria for study group

Presence of minimum of two of the following conditions were required to call it perinatal asphyxia:

- Indications of fetal compromise (such as heart rate less than 100 beats per minute, late decelerations or altered or absent beat to beat variability).
- Thick meconium stained amniotic fluid.
- Delayed cry at birth more than five minutes.
- APGAR score of four or less at one minute or six or less at five minutes.
- Active interventions at birth like requirement of positive pressure ventilation for more than a minute or need for oxygen post-delivery.
- Respiratory depression, convulsions, low muscle tone or bradycardia.
- Umbilical cord blood pH value below 7.2 with in 1 hour of delivery.

Inclusion criteria for control group

Neonates free of above inclusion criteria and with uneventful post-delivery course along with following were selected:

- Appropriate for gestational age after 37 weeks of gestation.
- APGAR more than seven at one and five minutes.
- Normal intrapartum heart rate.
- Clear liquor.

Exclusion criteria

Neonates with any of following were excluded from the study:

- Gross congenital defects including congenital heart defects.
- Rh factor incompatibility.
- Maternal drug abuse.
- Infant born to diabetic mothers.
- Preterm and post-term newborns.
- Non-availability of the consent.

Soon after birth, two samples of the cord blood were taken after cutting and clamping of cord. First sample was a heparinized sample which was immediately analyzed by blood gas analyzer for cord blood pH estimation. The second sample of cord blood was collected in EDTA coated vials for preparing smears. Two smears were made by standardized methods and stained with Leishman's stain. NRBC counts per 100 White blood cells were recorded in pathology lab of the college.

Other details of antenatal and intrapartum events were recorded in predesigned format. Clinical examination of the baby including neurological examination and detailed anthropometry was done. Newborns requiring NICU admissions were treated as per protocols. Neonates were categorized either into neurologically normal, abnormal or expired before discharge as the final outcome.

Then these babies were followed- up for one year to monitor their neurodevelopmental outcome. Follow- up visits were done at 3 months, 6 months, and 12 months. Detailed anthropometry was done at each visit and developmental assessment was done using Developmental Assessment Scales for Indian Infants (DASII) and entered into proforma. Motor and mental scores were computed separately. An infant scoring less than 70 in DASII was labeled as delayed.

The infants found to be having delay were appropriately put on early interventions like physiotherapy, ophthalmologic and auditory management.

Statistical analysis

Analysis of the collected data was done using descriptive statistics and hypothesis testing. The data was analyzed using SPSS for Microsoft windows 16.0. ANOVA and chi square tests were used for finding associations between various variables and strength of relationship. P value less than 0.05 was considered statistically significant.

RESULTS

Authors recruited 120 neonates (60 each of cases and controls) in present study and followed them up for 1 year. The flow chart of follow-up assessment is given in Figure 1.

There was statistically no significant difference in maternal age, gestational age, birth weight, and sex of cases and control groups (Table 1). But both these group had statistically significant differences among the frequency of cord complications, meconium staining of liquor, incidence of fetal distress, and APGAR scores at 1 and 5 minutes (Table 1).

Nucleated RBCs/100 WBCs were categorized into 3 groups for better understanding 0-10, 11-20 and >20. In the control group, 55 neonates had NRBCs 1-10, 5 had 11-20 and none had more than 20 with a mean value of

5.57±1.978. Cases were further divided into HIE stage I, II and III (as per Sarnat and Sarnat staging of Hypoxic-Ischemic Encephalopathy). 2 out of 22 cases of HIE stage I had 1-10 NRBCs while remaining had value between 11-20 and none had > 20 with total mean of 12.91±2.975. All newborns with HIE stage II (n=30) and HIE stage III (n=8) had NRBCs > 20 with a mean of 23.33±3.977 and 34.62±5.466 respectively (Table 2).

Developmental assessment of controls at 1st follow-up visit (n=60) showed that 9 had lost to follow-up and 1

had died of unknown cause, 46 out of remaining 50 had NRBC of 1-10, and 4 had 11-20/100 WBCs. On subsequent follow-up, 10 more lost to follow-up, all the remaining 40 newborn had normal development. Where as in case group, 22 patients had HIE stage I (2 with NRBCs of 1-10 and 20 with 11-20) of which 1 expired of unknown cause before first follow-up, 20 had normal development and 1 had mild motor delay by 3 months. On second follow-up visit at 6 months, 5 patients had lost to follow-up and 1 still had mild motor delay. But on final follow-up visit at 12 months, none had any delay.
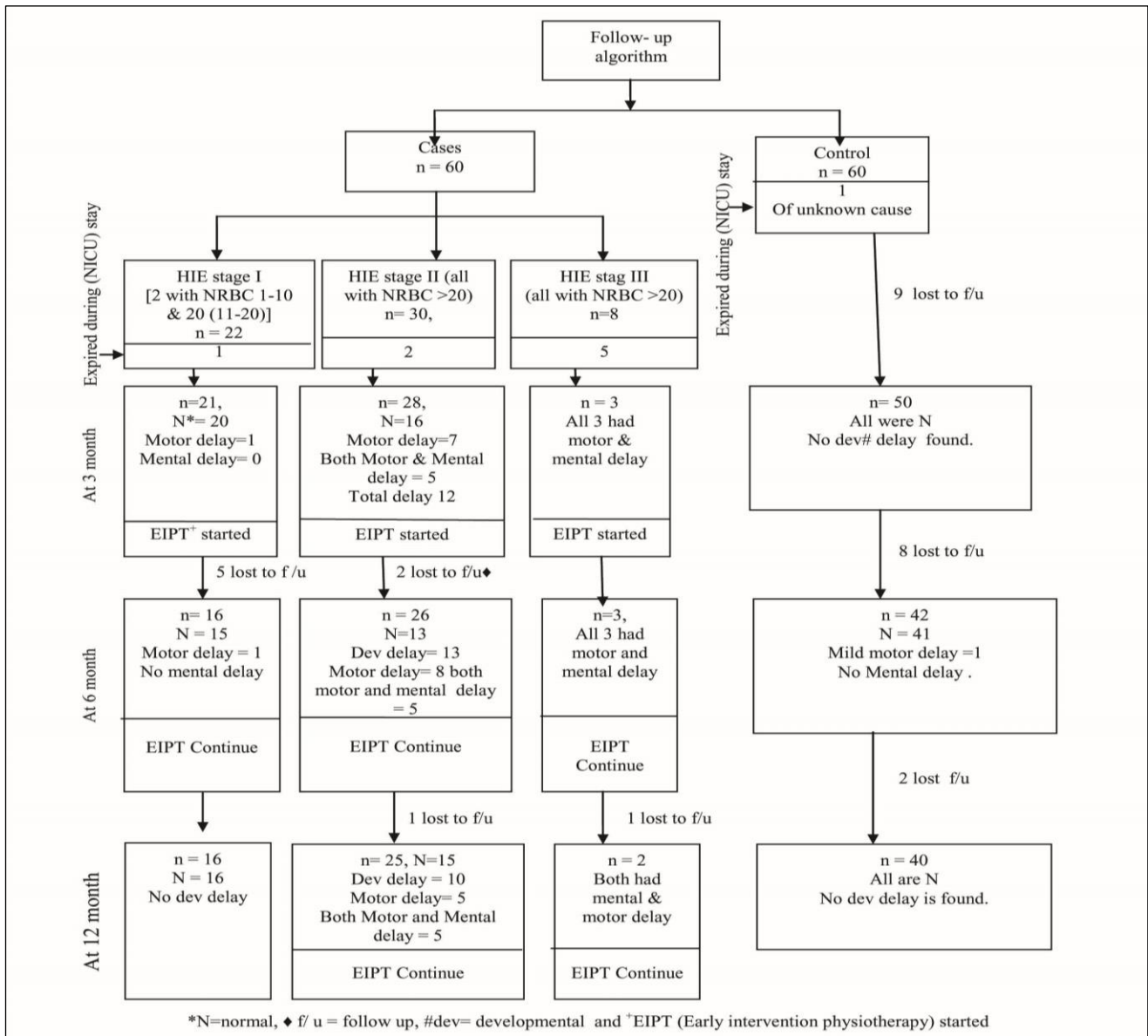

Figure 1: Follow-up algorithm developmental assessment.

All patients with HIE-II (n=30) had NRBCs >20/100 WBCs. At 3-month follow-up, 2 (6.67%) had expired, of the remaining 28 (93.33%) cases, 12 (42.85%) had

developmental delay. All were put on Early Intervention Physiotherapy (EIPT). At 6-month follow-up visit, 2 had lost to follow-up, and now 13 patients (50%) had

developmental delay despite EIPT. But at final follow-up visit at 12 months, 1 had lost to follow-up, and 10

patients (40%) had delay and 15 (60%) out of 25 patients were normal.

Table 1: Clinical details of study population.

	Control n=60	Cases n=60	P-value n=120
Maternal age (years)	23.67±2.98	23.3±2.58	0.4729
Cord complications	6	23	0.0005
Meconium liquor	12	32	0.0003
Fetal distress	8/60	34/60	<0.0001
Mode of delivery (NVD/ LSCS/ Forceps/ Vacuum)	40/18/2/0	22/30/6/2	-
Gestational age (weeks)	39.53±1.24	39.10±1.17	0.0519
Birth weight (gm)	2803±318	2643±552	0.0542
Sex (M/F)	34/26	36/24	0.8532
APGAR at 1 min	7.84±0.702	3.80±0.605	<0.0001
APGAR at 5 min	9.23±0.722	6.03±0.956	<0.0001
Cord blood pH	7.3040±0.0655	7.0158±0.2400	<0.0001
NRBCs/100 WBCs at birth	5.57±1.98	20.97±8.17	<0.0001

NVD- Normal Vaginal Delivery, LSCS- Lower Segment Caesarian Section, NRBCs- Nucleated Red Blood Cells, WBCs- White Blood Cells.

Table 2: Developmental assessment at the age of 3 months, 6 months and 12 months with respect to NRBC counts at birth in cases and controls.

Time of follow-up	Group	NRBCs/ 100 WBCs at birth	Only motor delay	Both motor and mental delay	No delay
3rd month	Control (n=50)	1-10[a,c]	0	0	46
		11-20[a,b]	0	0	4
	HIE stage I (n=21)	1-10[a,c] (n=2)	0	0	2
		11-20[a,b] (n=19)	1	0	18
	HIE stage II (n=28)	>20[b,c]	7	5	16
	HIE stage III (n=3)	>20[b,c]	0	3 (gross delay)	0
6th month	Control (n=42)	1-10[d,f]	0	0	41
		11-20[d,e]	1	0	1
	HIE stage I (n=16)	1-10[d,f]	0	0	1
		11-20[d,e]	1	0	14
	HIE stage II (n=26)	>20[e,f]	8	5	13
	HIE stage III (n=3)	>20[e,f]	0	3	0
1 year	Control (n=40)	1-10[g,i]	0	0	38
		11-20[g,h]	0	0	2
	HIE stage I (n=16)	1-10[g,i]	0	0	1
		11-20[g,h]	0	0	15
	HIE stage II (n=25)	>20[h,i]	5	5	15
	HIE stage III (n=2)	>20[h,i]	0	2	0

[a]P value=0.3239 when outcome compared between NRBC 1-10 and 11-20; [b]P value=0.0006 when outcome compared between NRBC 11-20 and >20; [c]P value< 0.0001 when outcome compared between NRBC 1-10 AND >20; [d]P value=0.0823 when outcome compared between NRBC 1-10 and 11-20; [e]P value=0.0047 when outcome compared between NRBC 11-20 and >20; [f]P value< 0.0001 when outcome compared between NRBC 1-10 AND >20; [g]P value=1 when outcome compared between NRBC 1-10 and 11-20; [h]P value=0.0012 when outcome compared between NRBC 11-20 and >20; [i]P value< 0.0001 when outcome compared between NRBC 1-10 AND >20

All newborns with HIE stage-III (n=8) had NRBC counts >20/100 WBCs. On first follow-up visit at 3 month, 5 (62.5%) had expired, and the remaining, 3 (37.5%) had gross delays. On second follow-up visit at 6 month, all 3 still had delays despite EIPT. On final follow-up visit at 12-month, 1 patient lost to follow-up and the remaining 2 still had gross delays. Upon testing for statistical significance, it was found that though cord blood NRBC count was a good marker of asphyxia at birth, but it had no influence on the final neurodevelopmental outcome as did HIE staging, therefore authors finally conclude that cord blood NRBC is not a good predictor of long term sequelae in case of perinatal asphyxia (Table 3).

Table 3: Final follow-up results at 1-year follow-up.

Stage of HIE	No. of patients	Normal	Delay
HIE-I (NRBCs <20)	16	16	-
HIE-II (NRBCs >20)	25	15	10
HIE-III (NRBCs >20)	2	-	2

P value <0.05 when outcome compared with NRBCs >20 between HIE-II and HIE-III with the poor outcome; P value <0.001 when negativity compared between NRBCs using Z proportion test.

DISCUSSION

In the present research work, apart from finding a relation between nucleated RBC counts at birth and perinatal asphyxia, authors made an effort to follow- up these patients till one year of life and study the correlation between the long-term neurodevelopmental outcome in these patients with respect to their NRBC counts at birth. While the maternal age, birth weight and sex of newborns did not significantly vary among cases and controls, authors found cord complications, meconium staining of liquor and incidence of fetal distress to be significantly associated to occurrence of birth asphyxia. Also, newborns with birth asphyxia and higher number of nucleated RBCs had significantly poor APGAR score at 1 and 5 minutes when compared to normal neonates. This is similar to the result of earlier study by Boskabadi et al.[9] There was a strong inverse correlation between umbilical cord blood pH and NRBCs at birth. Studies by Thilaganathan et al and Saracoglu et al suggested the same.[6,10]

Most of the previous studies which were on correlation between nucleated RBC with birth asphyxia such as Saracoglu et al, Phelen et al and Dasari et al reported a significant difference between their numbers seen in normal newborns and asphyxiated newborn being higher in the latter.[6-8] Present study also had similar results. When compared, the mean value of NRBCs/100 WBCs at birth was 5.57 ± 1.98 in normal neonates and 20.97 ± 8.17 in patients with birth asphyxia (P value less than 0.0001).

There are not many studies on association of NRBCs at birth with long term sequelae in a perinatal asphyxia. Phelan et al in their retrospective study suggested poor outcome among asphyxiated newborns with higher NRBCs at birth.[11] Walsh et al in their 2 year follow- up study have similar opinion and has advocated strongly to use nucleated RBCs at birth because it can be detected easily by microscope and require low cost.[12] Shivprakash and Nigam 2013 conducted a study to predict the occurrence of HIE by nucleated Red blood cells and creatinine kinase in cord blood of asphyxiated babies and assessment of outcome by follow up upto 6 months.[13] Anthropometry was done at birth, 1st month, 3rd month and 6th month (final visit). On follow- up in the final visit,

8 cases had motor delay and 7 cases had mental delay against no developmental delay in controls. They suggested that prediction of HIE in the asphyxiated cases could be done using the cord blood NRBCs and Creatine kinase, and suitable interventions and intensive monitoring could be planned thereby helping in identifying the high-risk cases. Authors too made attempt to see if NRBCs at birth is a good predictor of long term sequelae in asphyxiated neonates. Authors found that it was HIE staging at birth (HIE-III > HIE-II) which clearly determined the poor neurodevelopmental outcome and not NRBC count, as the latter was > 20/100WBC in both HIE-II and HIE-III stages, Authors found that it was HIE stage-III, which saw 62.5% mortality against 6.67% in stage II. Also, at 1-year follow-up all those (100%) who survived with HIE-III had gross developmental delay despite Early Intervention Physiotherapy against stage II survivors in which 60% eventually had normal neurodevelopmental outcome and 40% had some form of delay.

CONCLUSION

The present study concludes that simple estimation of NRBCs in the cord blood can help us distinguish an asphyxiated from a non-asphyxiated term neonate rapidly. In resource-poor countries, NRBC counts can be a useful part of the obstetrician's armamentarium for the evaluation of perinatal asphyxia where facilities for pH sampling are not available and can serve as a reliable, inexpensive and easily available marker of perinatal asphyxia. The present study differs from many such previous studies with respect to the follow-up of the newborns. Authors tried to study the relationship between the occurrence of developmental delay in asphyxiated newborns with respect to their cord blood NRBC counts at birth. Authors conclude that it is HIE staging at birth (HIE-III > HIE-II) which clearly determines the mortality and long-term morbidity in asphyxiated newborns at birth than their NRBC counts alone.

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