Original Research Article

Case control study of nucleated RBC's in cord blood as a predictor of perinatal asphyxia its severity and outcome

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ABSTRACT

Background: Perinatal asphyxia word derived from the greek word a-spyxos, meaning born without an evident pulse, is one of the most important causes of fetal distress. Inspite of major advances in technology and knowledge of fetal and perinatal medicine, it is one of the significant causes of mortality and long-term morbidity. World health organization (WHO) has defined perinatal asphyxia as a failure to initiate and sustain breathing at birth. HIE is one of the most common complication in an asphyxiated neonate because of its serious longterm neuromotor sequalae among the survivors. A detailed classification of HIE staging in term neonate was proposed by Sarnat and Sarnat.
Methods: The present study was prospective case control study conducted in neonatal intensive care unit of Department of Pediatrics at SGRD institute of medical sciences and research over a period of one year from September 2016 to august 2017. Total of 100 newborns among which 50 asphyxiated babies were designated to case group and rest 50 normal term babies to control group. The NRBC count of the case and control groups is compared. The NRBC's of subjects belonging to different stages of HIE is then compared. The results were analysed statistically chi-square analysis for variance (qualitative analysis), t-test (compare mean NRBC's in different stages) by SPSS version 20 software for biostatistic and p-value of <0.05 was considered statistically significant.
Results: Among total 100 babies included in the study, the male and female distribution was 22 (44%) and 28 (56%) in cases and 23 (46%) and 27 (54%) in controls respectively. In our study, the NRBC /100 WBC count for normal newborn was 0.88±1.35 and in case group it was 21.40±20.
Conclusions: In present study, the cord blood NRBC count was shown to be a good predictor of perinatal asphyxia with sensitivity of 86%, specificity of 100%, positive predictive value of 100% and negative predictive value of 87.72%. NRBC's can be used for early detection of HIE and its grading in asphyxiated neonates.

Keywords: HIE, Neonates, NRBC's, Perinatal asphyxia, Term

INTRODUCTION

Perinatal asphyxia word derived from the greek word a-spyxos, meaning born without an evident pulse, is one of the most important causes of fetal distress.[1]

Inspite of major advances in technology and knowledge of fetal and perinatal medicine, it is one of the significant causes of mortality and long term morbidity.

Perinatal asphyxia is a serious problem globally. It is a major cause of mortality and morbidity in newborns. Data from neonatal perinatal database suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India.[2] A gold standard definition of birth asphyxia does not exist. It is thus appropriate to use perinatal asphyxia as asphyxia may occur in utero, during process of labour, at birth, in postnatal period. World health organization (WHO) has defined perinatal

asphyxia as a failure to initiate and sustain breathing at birth.[3]

National neonatal perinatal database defines moderate asphyxia as a slow gasping breathing on APGAR score of 4-6 at 1 min of age and severe asphyxia was defined as no breathing on an APGAR score of 0-3 at 1 min of age. Perinatal asphyxia results in hypoxic injury to various organs including kidney, lungs, liver and most serious effects are seen on central nervous system.

Hypoxic ischemic encephalopathy refers to CNS dysfunction associated with perinatal asphyxia. The clinical features include altered consciousness, tone variability, seizures, autonomic disturbances and abnormalities of brain stem reflexes.

HIE is one of the most common complication of perinatal asphyxia because it causes serious longterm neuromotor sequalae among the survivors. A detailed classification of HIE staging in term neonate was proposed by Sarnat and Sarnat.[4] Parameters that have been used to predict or define perinatal asphyxia include-intrapartum electronic fetal monitoring, fetal or umbilical cord pH measurement, meconium stained amniotic fluid, apgar score, HIE and major organ disorder.

However, no single marker of perinatal asphyxia has shown good predictive efficiency and only combination of various indices can help in early diagnosis of perinatal asphyxia. Nucleated RBC's are commonly seen in the blood of neonates. NRBC counts in umbilical venous blood of neonates has been reported as a possible marker of perinatal asphyxia.[5]

NRBC's are primarily produced in fetal bone marrow in response to erythropoietin and are stored as precursors to reticulocytes and mature erythrocytes. Many acute and chronic stimuli cause an increase in the number of circulating NRBC'S from either an increase in erythropoietin activity or a sudden release from marrow storage pools.

It has been observed that hypoxic event induces compensatory response in the form of exaggerated erythropoiesis resulting in the release of immature RBC's in fetal circulation. The levels of NRBC's may be correlated with the presence of perinatal asphyxia.

Various studies have been conducted to calculate number of NRBC in cord blood in cases of perinatal asphyxia as it is a simple test and can be easily conducted even at primary health centres which are the backbone of health care system in india which may help in easy and early diagnosis of perinatal asphyxia.[6,7] This study aims to find values of NRBC's in normal and asphyxiated newborns and correlation of number of NRBC's in asphyxiated newborn and clinical outcome of such babies. It aims to study the role of NRBC's as early predictor of perinatal asphyxia, its severity and short term outcome.

METHODS

The present study is a prospective case control study conducted in neonatal intensive care unit of Department of Pediatrics at SGRD institute of medical sciences and research over a period of one year from September 2016 to August 2017.

Total of 100 newborns among which 50 asphyxiated babies were designated to case group and rest 50 normal term babies to control group.

Neonates born at 37-42 weeks with thick meconium staining of liquor, signs of fetal distress, apgar score of 4 at 1min, umbilical cord pH <7, those who required resuscitation for more than 1 minute with positive pressure ventilation were included in the study as cases.

Non asphyxiated newborn born at 37-42 weeks with apgar score >7 at both 1 and 5 mins, clear amniotic fluid and normal intrapartum fetal heart pattern with normal neurological evaluation were included in control group.

Newborns born to mother with h/o diabetes, eclampsia/ pre-eclampsia, Rh-sensitisation, chorioamnionitis, congenital TORCH infections, born at preterm or post term, twin pregnancy, birth wt <2500 gm, with any congenital anomaly like congenital heart diseases, haemolytic jaundice were excluded from study.

Immediately after birth, 2ml of the umbilical cord blood was collected in plain vial among both case and control group. Another 2 ml blood was collected in EDTA vial for routine haematological investigations to evaluate nucleated RBC's. Sample was assessed using BECKMAN COULTER machine and simultaneously a slide was prepared which was stained with leishman stain, slide was then examined under oil immersion lens for NRBCs/100 WBC count. Cord blood pH was also noted using Indikrom papers of Fisher Scientific brand (pH from 2-10.5) to detect pH from serum, according to color of indicator.

As soon as baby was admitted to NICU, the details were entered in predesigned proforma which included detailed history regarding antenatal risk factors to perinatal asphyxia; age of mother, history of pregnancy induced hypertension, anaemia, bleeding, intrapartum factors like mode of delivery, h/o prolonged rupture of membrane, meconium stained liquor and malpresentation.

Examination findings included vitals and detailed anthropometry with complete neurological and other system examination of the newborn. Daily evaluation for detection of abnormal signs and symptoms was done. Grading of neonates according to Sarnat and Sarnat for HIE was done. Correlation of cord blood NRBC's/100 WBC with clinical condition at different stages of HIE in NICU was observed until discharge or death of neonate.

The NRBC count of the case and control group is compared. The count of subjects belonging to different stages of HIE is then compared.

The results were analyzed statistically using chi-square analysis for variance (qualitative analysis), t-test (to compare mean NRBC in different stages) by SPSS version 20 software for biostatistic and p-value of <0.05 was considered statistically significant.

RESULTS

Among total 100 babies included in the study, the male and female distribution was 22 (44%) and 28 (56%) in cases and 23 (46%) and 27 (54%) in controls respectively. The most common mode of delivery was LSCS that is 28 (56%) in case group whereas in control group 28 (56%) delivered normally which was not statically significant. There was no significant difference between case and control groups in terms of mode of delivery and sex of the newborn. However, there was a statistically significant difference in birth APGAR at 1 minute and cord blood pH leading to HIE in case and control groups as shown in the Table 1.

Table 1: Various parameters of study population.

Parameters	Asphyxia (Case)	Control	P value
Gender (M/F)	22/28	23/27	>0.05
Mode of delivery (ND/CS)	22/28	28/22	>0.05
APGAR@1min	<7	>7	<0.05
CORD blood pH	<7	>7	<0.05

The NRBC/100 WBC count for case group was 21.40±20.31 with range from 8-100, whereas it was 0.88±1.35 with range from 0-4 in normal control group. t value was – 7.130; p <0.001 which was statistically, highly significant (Table 2).

Table 2: Distribution of NRBS 's per 100 WBC count in both groups.

nRBC	Non-Asphyxia	Asphyxia
0-10	50 (100%)	7 (14%)
11-50	-	39 (78%)
51-100	-	4 (8%)
Total	50	50
Range	0-4	8-100
Mean±SD	0.88±1.35	21.40±20.31

't' value = 7.130; p <0.001; Highly significant

The NRBC / 100 WBC count was correlated with degree of asphyxia and staging of HIE. The NRBC count / 100 wbc was found to be 12.33 (range 8-16) in stage 1 HIE, 22.50 (range 16-30) in stage 2, 85.0 (range 70-100) in stage 3 HIE. There was significant relationship between NRBC /100 WBC and HIE staging (P values <0.001) Table 3.

Table 3: NRBC/100 WBC in different HIE stage.

HIE Stage	NRBC	Range	NRBC Mean±SD
Stage I	30	8-16	12.33±2.38
Stage II	16	16-30	22.50±5.63
Stage III	4	70-100	85.00±17.32
Total	50	8-100	21.40±20.31

P<0.001; Highly significant

Table 4: HIE staging and outcome in cases.

Mortality	N	Range	NRBC Mean±SD
Alive	48	8-100	19.38±18.03
Death	2	70-100	70.00±0.000
Total	50	8-100	21.40±20.31

P<0.001; Highly significant

Among asphyxiated group, that is 50 cases 96% (48) had good outcome (Table 4 and 5) whereas only 2 cases had poor outcome i.e death.

Table 5: Relation of NRBC /100 WBC count with outcome.

HIE Stage	Alive	Death	Total
Stage I	30 (100%)	-	30
Stage II	16 (100%)	-	16
Stage III	2 (50%)	2 (50%)	4
Total	48	2	50

P <0.001; Highly significant

The NRBC/100 WBC count was significantly higher in babies with adverse outcome. than compared with babies who had good outcome (Table 5).

DISCUSSION

In this study, we tried to evaluate the relation of cord blood NRBC /100 WBC in predicting perinatal asphyxia and its immediate outcome. NRBC's are present in the placental vessels throughout first half of pregnancy but are uncommon later in pregnancy and are usually absent or present only in few numbers at term. Many conditions can be associated with increase of NRBC s in neonate but Fox et al found that acute asphyxia was the most common cause.[8]

In present study, the NRBC /100 WBC count for normal newborn was 0.88±1.35 and in case group it was 21.40±20.31. Several other studies have also reported an increased NRBC in neonatal cord blood following perinatal asphyxia. Gupta et al in their study had found out NRBC /100 WBC count of 5.7±2.33 in control group and 10.34±3.87 in asphyxiated group.[9] Previous studies suggested that erythropoietin increases erythroid production and release of nucleated erythrocytes into peripheral circulation in response to hypoxia.[10]

NRBC/100 WBC count was also related to Sarnat and Sarnat grading of HIE. We found out the higher value of NRBC/100 WBC count with higher degree of severity of HIE, 8-16 in stage 1 HIE, 16-30 in stage 2 HIE and 70-100 in stage 3 HIE. This is significant, as p value is <0.001. Hermansen et al, Phelan et al and Hanlen-Lundberg et al had also found higher number of cord blood NRBC's in case of severe asphyxia.[11-13] Boskabadi et al in their study had foung higher NRBC counts as follows, 11.94 in HIE stage 1, 21.08 in HIE stage 2 and 29.18 in HIE stage 3.[14]

In present study, among asphyxiated babies 4% (2) had poor outcome, either death or survival with sequelae. In these babies NRBC count was higher that is more than 50 whereas babies with good outcome had low NRBC count, i.e less than 50, which is found to be statistically significant. Similar correlation between the control and case group was reported by Shivprakash et al and Fern's et al.[15,16] Phelan et al showed that all the neonates with neurological impairment secondary to perinatal asphyxia had significantly higher number of NRBC/100 WBC than control (p value <0.0001).[12]

Mohammed et al reported that increase in number of NRBC's is an early marker to detect asphyxia and subsequent neurological impairment and stages of HIE.[17] Hence from present study we conclude that umbilical cord NRBC'S/100WBC counts can be used as a marker for perinatal asphyxia.

CONCLUSION

In present study, the cord blood NRBC count was shown to be a good predictor of perinatal asphyxia with sensitivity of 86%, specificity of 100%, positive predictive value of 100% and negative predictive value of 87.72 %. NRBC's can be used for early detection of HIE and its grading in asphyxiated neonates. As, it is a simple, cheap, rapid and non-invasive test of NRBC Count Obtained from cord blood, it can be easily made available as a marker to evaluate perinatal asphyxia, predicting its severity, especially in developing nations like our's, where blood gas analysis facilities are not available at majority of places.

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