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CORD BLOOD NUCLEATED RED BLOOD CELLS - A MARKER OF FETAL ASPHYXIA

Gynecology

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ABSTRACT

Objectives: To study the cord blood nucleated red blood cell count (NRBC) in asphyxiated and non asphyxiated fetuses at birth and to find out the correlation between NRBC count, fetal acidosis and various clinical markers of asphyxia.

Material and methods: A prospective, comparative case-control study was undertaken in a tertiary care hospital from January 2008 to November 2009, which included a total of 200 neonates with 100 asphyxiated babies (case group) and 100 normal babies (control group). Parturient women in labor between 37-40 weeks of gestation were selected after satisfying inclusion and exclusion criteria and then divided in 2 groups. The cord blood was collected immediately after delivery for measurement of NRBC/100 WBC, and pH. Statistical analysis was performed with chi-square, Mannwhitney U-test and student t-test.

Results: The mean NRBC count was higher (23.3 ±10.1) in cases than in controls, 12.5 ±4.7 ($p < 0.05$) The NRBC count was significantly higher in MSAF, in fetuses with non-reassuring fetal heart pattern and those with apgar score < 6 at 5 minutes ($p < 0.05$).

Conclusion: NRBC counts of the cord blood can be used as an effective tool to confirm perinatal asphyxia. It is a simple, quick, accurate and clinically effective test to diagnose and initiate treatment to prevent long term sequel of perinatal asphyxia.

KEYWORDS

Cord blood nucleated RBCs, Perinatal asphyxia

Introduction

Perinatal asphyxia is a major cause of acute mortality and chronic neurologic disability amongst survivors, and is a complication that occurs between 2-10% of deliveries.[1] The complications of perinatal asphyxia are many like respiratory distress syndrome, necrotising enterocolitis, hypoglycemia, hypocalcemia, meconium aspiration syndrome etc. but the most severe being Hypoxemic ischemic encephalopathy (HIE) and multiorgan failure.[2] Of patients with HIE, 15-20% die in the neonatal period, and 25-30% of the survivors develop permanent neurological disorders, such as cerebral palsy (CP) and mental retardation.[3] Perinatal asphyxia at birth is best confirmed by measurement of acidosis in cord blood[1] but it is not always possible to asses scalp blood or cord blood pH when adequate facilities are not available. Currently the clinical surrogate markers like meconium stained amniotic fluid (MSAF), non reassuring fetal heart patterns and low apgar scores are being used as the predictors of fetal acidosis. But the correation between these clinical markers and acidosis was found to be unsatisfactory.[4] In situations where facilities for cord bood gas analysis are not available it would be very useful to have another surrogate marker for perinatal asphyxia. Considering the haemapoietic response to hypoxia in utero the elevated count of nucleated red blood cell (NRBC) was investigated recently as a possible marker of asphyxia.[5]

Our study aimed to study the cord blood nucleated red blood cell count in asphyxiated and non asphyxiated fetuses at birth and to find out the correlation between NRBC count, fetal acidosis and various clinical markers of asphyxia.

Material and Methods

This was a prospective comparative study conducted in the department of OBG, Lalla Ded Hospital, Government Medical College, Srinagar, from January 2008 to November 2009; informed consent was obtained from all subjects in the study. A total of two hundred subjects were taken in this study which included hundred cases (asphyxiated group) and hundred controls (nonasphyxiated). Inclusion criteria included neonates born to mothers who are primigravida having singleton pregnancy with gestational age 37-40 weeks in spontaneous labour. Neonates born to mothers with pre-eclamptic toxemia, diabetes mellitus, Rh-sensitization, multiple pregnancies, born by breech presentation, born before 37 weeks gestation and birth weight <2.5 kg were excluded from the study. For asphyxiated group two or more of the following criteria were chosen: thick meconium-staining of Liquor, Apgar at 5 minutes <6 and non reassuring fetal heart pattern.Control group included nonasphyxiated newborns with clear amniotic fluid, normal intrapartum fetal heart rate (FHR) pattern and Apgar score >6 at 5 minutes.

One ml of blood was collected in heparinized syringe from doubly clamped segment of umbilical cord for pH estimation and another 2 ml blood collected in ethylenediaminetetraacetic acid (EDTA) vial for the purpose of making a thin blood film on a glass slide, which was dried and stained with Leishman's stain. Under supervision of the haematologist, the dried smear was focused under high power of microscope and the number of NRBC were counted against the number of WBC until 100 WBCs were counted. The relationship between NRBC count and pH was analysed in both the groups. The correlation between MSAF, Non reassuring fetal heart pattern, low apgar score (the clinical markers of fetal asphyxia) and NRBC count was also analysed. Asphyxiated babies were transferred to the NICU (neonatal intensive care unit) after initial resuscitation. Normal babies were shifted to the ward. The infants were followed upto 8 weeks of age.

Statistical Analysis

The data was expressed as mean and percentage. The characteristics of cases and controls were compared by students t-test, Mannwhitney U-test, chi-square analysis and analysis of variance. Two tailed p value of <0.05 was considered significant.

Results

Both the case group and control group were similar in terms of maternal age, gestational age, mode of delivery, birth weight and sex of baby as shown in table 1.

Table 1: Various parameters of Study Population

Parameters	Case (asphyxiated)	Controls (nonasphyxiated)	p-value
Maternal age (years)	27.5± 4.2	26.9 ± 4.6	>0.05
Gestational age (weeks)	38.4 ±1.2	38.4 ± 1.1	>0.05
Mode of delivery (VD/CS)	52/48	65/35	>0.05
Birthweight (kilograms)	2.93 ± 0.48	3.04 ± 0.49	>0.05
Sex (M/F)	48/52	53/47	>0.05

Data presented as mean ± SD; VD= Vaginal Delivery; CS= cesarean Section

Table 2: Correlation between NRBC count and Clinical Markers of Asphyxia in Study Population

Clinical Markers of Asphyxia		Cases (n)	Controls (n)	Total Number	NRBC count (mean ±SD)	p-value
MSAF	Present	94	0	94	23.3 ± 10.2	<0.05
	Cear	6	100	106	13.1 ± 5.7	
Fetal Heart Rate Pattern	Reassuring	23	100	123	14.4 ± 7	<0.05
	Non-reassuring	77	0	77	23.6 ± 10.4	
Apgar Score at 5 minutes	>6	25	100	125	14.2 ±7.4	<0.05
	<6	75	0	75	24.0 ± 9.7	

Table 2 shows the distribution of clinical markers of asphyxia and their correlation with NRBC count in the studied population. The NRBC count was significantly higher in MSAF, in fetuses with non reassuring fetal heart pattern and those with apgar score < 6 at 5 minutes.

Table 3: Distribution of Umblical artery pH, base excess and NRBC count in Study Population

	Cases	Controls	P value
Umblical artery pH	6.94 ±0.08	7.22 ±0.56	<0.05
Base excess	-13.7 ±4.1	-3.7 ±3.7	<0.05
NRBC count	23.3 ±10.1	12.5 ±4.7	<0.05

The mean umblical artery pH and base excess in cases was significantly lower than that in the controls, while NRBC count was significantly higher in cases than that in controls as shown in table 3.

Table 4: NICU admission in Study Population

NICU Admission	Case (n)	Control(n)	P value
Yes	45.0	2.0	<0.05
No	55.0	98.0	

As shown in table 4, fortyfive neonates from asphyxiated group were admitted in NICU and only two from control required admission to NICU. The mean NRBC in neonates admitted in NICU was 29.5 ± 9.3, whereas in rest of the neonates, the mean NRBC count was 18.3 ± 7.6. This difference was statistically significant (p < 0.05).

Table 5: NRBC in various grades of HIE

HIE Grade	Number	NRBC range (mean±SD)	P value
Mild	10	26.2±10.3	> 0.05
Moderate	7	33.0±4.4	
Severe	4	35.8±6.6	

As shown in table 5, the NRBC count correlated with the grade of HIE. NRBC counts increased with the severity of HIE but the difference was statistically insignificant because of small sample size (21). Among 100 asphyxiated neonates there were 5 neonatal deaths and 21 neonates developed various grades of hypoxic-ischemic encephalopathy while 74 neonates had good outcome (survived with normal neurologic development).

Discussion

Although NRBCs rarely circulate in older children, they are commonly seen in the blood of neonates. In normal newborns less than 10 NRBC/100WBC is within normal limit.[6] it has been observed that hypoxia causes stimulation of the hematopoietic system as evidenced by the rise in levels of erythropoietin. This rise in erythropoietin levels translates into the release of erythropoid precursors into the circulation of the fetuses.[7]

In our study the mean NRBCs in asphyxiated neonates was 23.3± 10.1 when compared to 12.5±4.4 in non asphyxiated neonates. Similar results were reported in previous studies. In Dasari Papa et al study the mean NRBCs in acidotic neonates was 25.65± 10.14 and that in non acidotic fetuses was 12.33±5.51.[8] Phelan et al found the mean NRBCs of 3.4 ± 3.0/100 WBCs in non asphyxiated neonates and 34.5 ± 68.3 in asphyxiated neonates.[9] In the study of Hanlon-Lundenberg et al the NRBCs count in term singleton neonates was 8.55±10.27 and that in the study of Saracoglu et al was 7.56 ±3.85.[10,11]

Several authors have evaluated the relationship between various surrogate markers of asphyxia and NRBC count. Spencer et al concluded that NRBC count is a better marker of fetal metabolic acidosis than MSAF, nonreassuring FHR, low apgar scores, and fetal erythropoietin levels.[12] In our study the mean NRBC count was significantly higher in MSAF than in clear liquor. Similar observations were made by Dasari Papa et al and Redzko et al.[8,13] Dolberg et al found increased NRBC count in neonates with meconium aspiration syndrome.[14]

In our study the NRBC counts were significantly higher in fetuses with non-reassuring FHR pattern than those with normal FHR pattern. In the study of Ferber et al the median NRBC count was 9 in cases with non-reassuring FHR as compared with 5 in controls (p <0.005).[15] In Dasari Papa et al study, although the NRBC counts were higher in relation with the abnormal FHR pattern as compared to those with reassuring FHR pattern, the difference was statistically insignificant pattern.[8]

NRBC counts when analysed in relation to apgar scores were found to be inversely proportional to apgar scores at 1 and 5 minutes. Spencer et al found that NRBC count was a better marker of fetal metabolic acidosis than apgar scores. In our study the NRBC counts correlated with low apgar scores.[12] Dasari Papa et al made similar observation.[8] Phelan et al reported higher NRBC counts in neonates who suffered hypoxic ischemic encephalopathy and subsequent neurological impairment.[6]

NRBC/100 WBC was also related to Sarnat and Sarnat's grading of HIE.[16] In our study we found the higher values of NRBC/100 WBC count, in higher degree of severity of HIE. Boskabadi et al in their study had found NRBC as follows, 11.94 in Grade I HIE, 21.08 in Grade II HIE and 29.18 in Grade III HIE cases.[17] Other studies which showed significant correlation between increasing NRBCs count with severity of HIE are Hermansen et al, Goel et al and Ghosh et al.[18,19,20] They also reported that neurological impairment was directly related to the severity of asphyxia.

In our study 26 of 100 asphyxiated neonates had significant morbidity in the form of severe birth asphyxia and HIE and five of them expired. It was seen that neonates with poor outcome had higher counts of NRBC than those with better neonatal outcome.

Conclusion

Present study found a strong relationship between birth asphyxia and raised NRBC counts. NRBC count can be used as an effective tool to diagnose perinatal asphyxia. In low resource settings of developing countries like India where costly and invasive tests like scalp PH determination and fetal ABG analysis to assess fetal acidemia are not easily available. The determination of NRBCs from the cord sample in suspected cases of perinatal asphyxia can be used as a simple, easily available, rapid and inexpensive test to confirm and to initiate treatment early to prevent mortality and long term morbidity.

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