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Original Article

Cord blood nucleated red blood cell count - a marker of fetal asphyxia

Dasari Papa [1], Phani Jyotsna G [1], Badhe Bhawana Ashok [2]

[1] *Department of Obstetrics and Gynecology, JIPMER,* [2]*Department of Pathology, JIPMER*

Abstract

Objectives : To study the cord blood nucleated RBC count (NRBC) in asphyxiated and nonasphyxiated fetuses at birth and to find out the correlation between NRBC count, fetal acidosis and the clinical markers of asphyxia. *Methods :* This is a prospective comparative study conducted between 2002 and 2004. Parturient women who were in labor between 37 and 42 weeks of gestation were selected after satisfying inclusion and exclusion criteria and divided in 2 groups. The control group consisted 51 cases with nonasphyxiated fetuses and study group 52 asphyxiated fetuses. The cord blood was collected soon after birth, one sample for pH determination and the other sample for making smears that were stained with Leishman's stain. NRBCs were counted against 100 WBCs. The statistical analysis was done using SSPS and Chi-square test to find out the relationship between pH and NRBC count and the correlation between NRBC count, and meconium stained amniotic fluid (MSAF), nonreassuring fetal heart rate pattern and low apgar scores. *Results :* The mean NRBC count in the study group was 25.65 ± 10.14 as compared to 12.33 ± 5.51 in the control group (p=0.003). The NRBC count was significantly higher in MSAF and in neonates with low apgar scores of ≤ 6 at 5 minutes (P=0.02). *Conclusion :* Acidotic neonates (cord blood pH ≤ 7.1) at birth had higher mean NRBC count compared to nonacidotic neonates and hence NRBC count of cord blood smear serves as a good indicator of fetal asphyxia.

Key words : cord blood nucleated RBCs, fetal acidosis

Introduction

Fetal asphyxia at birth is best confirmed by the measurement of acidosis in cord blood[1] but it is not always possible to assess scalp blood or cord blood pH when adequate facilities are not available. Currently the clinical surrogate markers like thick meconium stained liquor, nonreassuring fetal heart patterns, and low apgar scores are being used as predictors of fetal acidosis. But the correlation between these clinical markers and acidosis was found to be unsatisfactory [2]. In situations where facilities for cord blood gas analysis are not available it would be very useful to have another surrogate marker for fetal asphyxia. Considering the hematopoietic response to hypoxia in utero the elevated count of nucleated red blood cells (NRBCs) was investigated recently as a possible marker of asphyxia[3]. This study attempted to examine the relationship between NRBC count and fetal acidosis, and current clinical markers of asphyxia.

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Correspondence :
Dr. Papa Dasari
Professor
Department of Obstetrics & Gynaecology,
JIPMER,
Pondicherry - 605 006.

Methods

This was a prospective comparative study conducted during the period of December 2002 to February 2004.

The study group included 52 and control group 51 pregnant women who were in labor between 37 and 42 weeks of gestation and satisfied the inclusion and exclusion criteria. For the study group two or more of the criteria, viz, thick meconium stained amniotic fluid (MSAF) nonreassuring fetal heart rate (FHR) pattern and low apgar scores i.e. ≤ 6 at 5 minutes of birth were chosen. For the control group, criteria were presence of clear amniotic fluid, reassuring FHR pattern and apgar scores of > 6 at 5 minutes of birth. Patients with Rh incompatibility, diabetes mellitus and postterm pregnancy were excluded. From all the subjects, two samples of cord blood were collected immediately after clamping and cutting the umbilical cord. One sample of umbilical arterial blood in a heparinized syringe was drawn from an isolated segment of cord for the purpose of estimation of pH. A second sample of mixed cord blood was taken in an EDTA coated bottle for the purpose of making smears.

Umbilical arterial blood pH was estimated by using a CIBA 280A blood gas system. For making smear two clean glass slides were taken and a drop of the sample was placed towards one end. A spreader glass slide was placed at 45^0 inclination to the sample and in one uniform motion the drop of blood was smeared onto the rest of the slide. The slide was allowed to dry and then covered with Leishman's stain. After 3 minutes the stain was carefully diluted with distilled water and mixed on the slide by gently blowing on the surface. The slide was allowed to take in the stain for 15 minutes and then washed in a gentle stream of tap water. Under supervision of a pathologist the dried smear was focused under high power of microscope and the number of nucleated red blood cells were counted against the number of white blood cells until 100 white blood cells were counted (Figure 1).

Statistical analysis was done using SPSS and Chi square test.

The relationship between NRBC count and pH was analysed in both the groups. The correlation between MSAF, nonreassuring FHR pattern, low apgar scores and NRBC count was also analyzed.

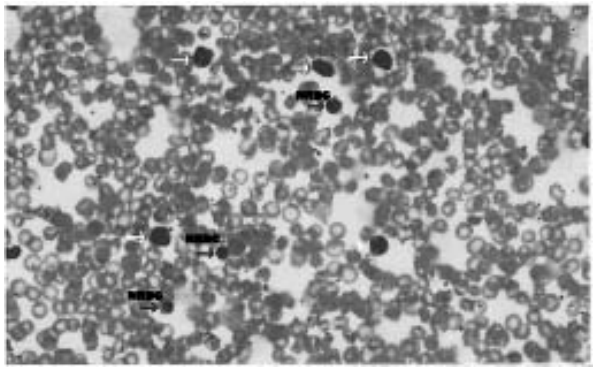

Figure 1. Photomicrograph of cord blood smear stained with Leishman's stain. 40X magnification. The red arrows represent NRBCs and white arrows WBCs on a background of normal fetal RBCs.

Results

There was no statistically significant difference in age and period of gestation in the two groups (Table 1)

Table 1. Patient profile

Characteristic	Study group	Control group	P Value
Mean age ± SD	24.02 ± 3.39	23.35 ± 3.67	0.982
Mean period of gestation ±SD	272.69±34.27	274.75 ± 7.45	0.150

Table 2. NRBC count and cord blood pH

Characteristic	Study group	Control group	P Value*
	pH ≤ 7.1 n=52	**pH > 7.1 n=51**	
Mean NRBC Count ± SD	25.65 ± 10.14	12.33 ± 5.51	0.003

*p <0.05 - Significant

The mean NRBC count in the study group was 25.65 ± 10.14 per 100 WBCs (range 11 to 58) and in the control group it was 12.33 ± 5.51 per 100 WBCs (range 5 to 28). This difference was statistically significant (p=0.003).

Table 3. NRBC count and clinical surrogate markers of fetal asphyxia

Clinical Marker	Number N	Mean NRBC Count ± SD	P value
Amniotic fluid			
Thick MSAF	41	27.41 ± 10.13	
Clear/Thin MSAF	62	13.53 ± 6.37	0.021
FHR pattern			
Non-reassuring	47	24.89 ± 9.49	
Reassuring	56	14.16 ± 8.78	0.519
Apgar at 5 minutes >6	47	26.23 ± 10.01	0.026
>6	56	13.04 ± 6.42	

Table 4 . NICU admission in study group

NICU admission	Number	Mean NRBC Count ± SD	P Value
Yes	30	29.80 ± 10.90	0.006
No	22	20.00 ± 5.26	

Correlation of NRBC count with clinical surrogate markers is shown in Table 3. The NRBC count was significantly higher in MSAF and in fetuses with apgar score ≤6 and 5 minutes. When NRBC count was compared between non-reassuring FHR and reassuring FHR, though higher count was observed in non-reassuring FHR the difference was not statistically significant.

There was no neonatal morbidity or mortality in the control group. In the study group, 30 out of 52 neonates had morbidity and the difference in the mean NRBC count in those who required NICU admission and in those not requiring it was statistically significant as shown in Table 3. The most common morbidity was respiratory distress. Four neonates developed seizures and three expired due to severe asphyxia. The photomicrograph of cord blood smear stained with Leishman's stain is seen in Figure 1.

Discussion

Various investigators have proposed that the intrauterine hypoxia caused to the fetus can set off a number of biochemical and hematological responses. 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 erythroid precursors into the circulation of the fetus[4].

NRBCs cells are quite common though their number varies[5,6]. Phelan et al[7] found the mean NRBCs of 3.4 ± 3.0 per 100 WBCs in non-asphyxiated neonates and 34.5 ± 68.3 in asphyxiated neonates. In our study the mean NRBCs in acidotic fetuses was 25.65 ± 10.14 when compared to 12.33 ± 5.51 in non acidotic fetuses. In the study of Hanlon-Lundenberg et al[5] the NRBC count in term singleton neonates was 8.55 ± 10.27 and that in the study of Saracoglu et al[6] was 7.56 ± 3.85.

Several authors have evaluated the relationship between various surrogate markers of asphyxia and NRBC count. Spencer et al[8] concluded that NRBC count is a better marker of fetal metabolic acidosis than MSAF, nonreassuring FHR, low apgar scores, and fetal erythropoietin levels. The mean NRBC count was higher in MSAF than in clear liquor and this was statistically significant in our study. Similar

observation was made by Redzko et al[9]. Dollberg et al[10] found increased NRBC count in neonates with meconium aspiration syndrome.

The median NRBC count was 9 in cases with nonreassuring FHR pattern compared to 5 in controls in the study reported by Ferber et al[11]. The presence of decelerations correlated best with raised NRBC. In our study, though there was no statistically significant correlation between NRBC count and abnormal FHR pattern, the mean counts were higher in nonreassuring FHR pattern than those in reassuring FHR pattern (24.89 ± 9.49 vs 14.16 ± 8.78).

NRBC counts when analyzed in relation to apgar scores were found to be inversely proportional to apgar scores at 1 and 5 minutes[2,3,5]. Spencer found that NRBC count was a better predictor of acidosis than apgar scores[8]. In our study the NRBC counts correlated with low apgar scores. Phelan et al[7] showed higher NRBC counts in neonates who suffered hypoxic ischemic encephalopathy and subsequent neurological impairment. In our study 15/52 (28.8%) of study group neonates had significant neonatal morbidity in the form of severe birth asphyxia, seizures or hypoxic encephalopathy and three of them expired. The clearance of the NRBCs from the circulation may be of help in prognosticating the outcome of these asphyxiated neonates as suggested by Korst et al[3]. Thus it can be concluded from this study that NRBC count correlates well with fetal acidosis in asphyxiated neonates and this is a simple bed side test that can be utilized to diagnose fetal acidosis in health centers where facilities for determining cord blood pH are not available.

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