Original Research Article

Nucleated RBCs in cord blood as a predictor of fetal asphyxia

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Abstract

Background: 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. No single parameter can define perinatal asphyxia, rather a combination of parameters like fetal distress, meconium-stained liquor, low Apgar score, umbilical cord blood pH and clinical features of hypoxic-ischemic encephalopathy (HIE) can predict it.
Aim: To study the correlation between nucleated red blood cell count (NRBC), fetal acidosis and clinical markers of asphyxia.
Materials and methods: 52 pregnant women with fetal distress as a study group and 51 pregnant women without distress as a control the ed group were selected and their cord blood was analyzed for pH and nucleated RBCs. The results were analyzed.
Result: Higher NRBC count was detected in the study group with signs of asphyxia (Thick MSAF, Non-Reassuring FHR Pattern, Low APGAR score).
Conclusion: NRBC count is a simple bedside test to diagnose fetal asphyxia. Since it is cost-effective and does not require any special expertise or any high-tech facilities, it may be a useful, reliable, inexpensive and easily available markan er to evaluate perinatal asphyxia, especially in a resource-poor country like ours, where blood gas analysis facilities are not available in the majority of place.

Key words

NRBC count, Perinatal asphyxia, Cord Blood.

Introduction

The goal of fetal surveillance is to decrease perinatal morbidity and mortality. Fetal asphyxia is best confirmed by acidosis in cord blood. Currently, markers like thick meconium stained liquor, nonreassuring fetal heart, low APGAR are used as predictors [1]. As the correlation between them is not good, we need more satisfactory markers of asphyxia. In response to asphyxia, there is stimulation of fetal hemopoietic system leading to increased erythropoietin and release of immature erythrocytes into fetal circulation [2]. This is reflected as nucleated RBC sand erythropoietin in cord blood. The process of erythropoiesis is dependent primarily on the action of erythropoietin on sensitive tissues in both the fetal and adult body. In the adult, the kidney is the organ producing erythropoietin but in the fetus, it is the liver, which synthesizes erythropoietin till near term when all erythropoiesis takes place in the bone marrow [3]. When there is a moderate degree of hypoxic insult lasting at least 2-4 hours, erythropoietin levels increase and set about the proliferation of erythroid series, leading to the release of many immature forms into the circulation [4]. In normal uncomplicated pregnancies at term, the fetal plasma erythropoietin levels range from 10-60 mU/ml. NRBCs are immature erythrocytes that are found in the circulation of neonates. It is believed that fetal asphyxia of either an acute or chronic type leads to the stimulation of the hematopoietic system by increasing levels of erythropoietin [5]. Philip AG, et al. studied NRBC counts in 46 singleton term neurologically impaired neonates in one study and 153 impaired neonates in a follow-up study, where the cause of the neurological damage was fetal asphyxia and found that there was a significant rise in NRBC counts in comparison to normal neonates [6]. They suggested that closer the asphyxial event was in time to the birth of the neonate; the smaller will be the rise in NRBC count and concluded that NRBC counts can be used as a marker for fetal asphyxia [7]. Indicators of fetal asphyxia:-Umbilical artery metabolic or mixed respiratory academia with pH less than 7.00.A persistent Apgar score of 0-3 for more than 5 minutes. Neonatal neurological sequelae such as seizures, coma, or hypotonia. Meconium in amniotic fluid has been considered to be a marker for fetal asphyxia for a long time. But later studies found that thick meconium, in comparison to thin meconium was the single most significant factor influencing the fetal outcome. Cord blood pH has been considered the gold standard for confirming the diagnosis of fetal asphyxia [8]. The merit of routine cord blood pH measurement was highlighted by Vandenbussche and he proposed to classify arterial cord blood pH into three categories: Normal (when>7.11), Abnormal (when<6.99), Borderline (7.00-7.11). More significant than considering pH in isolation would probably be to measure the base deficit and bicarbonate levels in umbilical cord blood [9]. A base deficit between 12 to 16 mmol/L was associated with significant metabolic acidosis. The median base deficit associated with morbidity was 19 mmol/L in comparison with 14 mmols/L in those without morbidity as found in the study done by Andres, et al. Apgar scores at 1 and 5 minutes tended to be inversely related to NRBC count. Significance reached in the 1-minute 0-3 Apgar score range [10]. Umbilical cord pH showed a trend towards inverse relationship to the NRBC. Cases in which cord blood pH was 7.10 to 7.19 had a significantly higher NRBC count than the reference range ($p<0.05$). Cases in which cord blood pH was 7.30 to 7.39 and 7.40 to 7.49 had significantly lower NRBC counts ($p<0.0001$). The association between abnormal fetal heart rate patterns and NRBC counts was studied in a univariate analysis by Ferber et al in 2003 and it was concluded that elevated NRBC counts were significantly associated with absence of accelerations and presence of decelerations [11]. The predictive values for Apgar scores, nonreassuring electronic fetal heart monitoring traces, meconium-stained amniotic fluid, fetal erythropoietin levels. It was proposed that not only can these nucleated RBCs be used as a marker of fetal asphyxia, they can also be used to time the asphyxial event [12]. It was found that

counts peaked 2 hours after the hypoxic injury and normalized in 24-36 hrs. Hence NRBC counts could be used to distinguish between acute hypoxia and chronic hypoxia [13]. The most feared consequence of fetal asphyxia is a permanent neurological injury. Phelan et al found that NRBC count was higher in neonates with hypoxic-ischemic injury and permanent neurological impairment. The count was higher in cases with pre-admission injury than in those with an acute injury [14]. Elevated NRBC count in cord blood or early neonatal blood was found to be a good marker, of neonatal brain damage in premature LBW neonates, who suffered from perinatal asphyxia and occurrence of early-onset neonatal seizures [15].

Materials and methods

This was a prospective comparative study done in Department of Obstetrics and Gynaecology, Rajah Muthiah Medical College Hospital to evaluate the role of NRBC count as a marker of fetal asphyxia. The study group included 52 pregnancies of 37 to 42 weeks with any 2 of 3 markers of asphyxia. Cord blood in all cases was less than 7.1. The control had 51 pregnancies of 37 to 42 weeks with clear liquor, reassuring for, normal apgar, cord blood >7.1. From all subjects, two samples of cord blood were collected. One in heparinised syringe of umbilical arterial blood for estimating Ph. Other mixed cord blood in EDTA for smears. The smear was dried and covered with Leishman Stain for 15 minutes and washed in tap water and checked for nucleated RBCs against 100 WBCs. The relationship between NRBC count and Ph analysed. The correlation between MSAF, non-reassuring fetal heart rate pattern. Low Apgar and NRBC count were analysed. statistical analysis done using chi-square test.

Results

The mean age and period of gestation in both groups were comparable. The parity in both groups was also comparable, though the majority were primigravidas.

The range of NRBCs in control was 5 to 28 per 100 WBC sand in the study was 11 to 58 per 100 WBCs. The acidotic fetuses clearly showed a significant rise in mean NRBC count (**Table – 1**).

Table – 1: Analysis of NRBC counts.

Characteristic	Study group N=52	Control group N=51	P value
Mean NRBC+ S.D	25.65	12.33	0.003

Table – 2: NRBC count and amniotic fluid.

Amniotic fluid	N	Mean NRBC count	P - value
Thick MSAF	41	27.41	0.021
Clear/ Thin MSAF	62	13.53	

Table – 3: NRBC count and FHR pattern.

FHR pattern	N	Mean NRBC count	P-value
Non reassuring	47	24.89	0.59
Reassuring	56	14.16	

Table – 4: NRBC count and APGAR.

APGAR	N	Mean NRBC count	P-value
AT 1' <6	50	26.00	
AT 1',6	53	12.51	0.003
AT 5',6	47	26.23	0.026
AT 5',6	56	13.04	

Table – 5: Neonatal outcome.

NICU admission	N	Mean NRBC count	P-value
YES	30	29.80	
NO	22	20.00	0.006

Cases with thick meconium had higher NRBC and the difference was statistically significant when compared to clear or thin MSAF (**Table – 2**).

Out of 52 acidotic cases, non-reassuring for was seen in 47 cases. The mean RBC count difference between the two groups was not statistically significant but the non-reassuring group had higher mean RBC count (**Table – 3**).

The 1 min and 5 min Apgar score of both groups were taken. It was found that low Apgar score correlated well with increased NRBC counts (**Table – 4**).

The neonatal outcome in the control group was very good. the mean NRBC Count was significantly higher in those who required NICU admission .the most common was respiratory distress and 4 neonates developed seizures.3 died due to severe birth asphyxia (**Table – 5**).

Discussion

Parturition is one of the most stressful periods the fetus has to undergo. Based on the hypothesis that hypoxia triggers off a hemopoietic response, the study was aimed to analyze NRBC counts in relation to fetal asphyxia. There was no significant difference in age, parity and period of gestation in both cases and controls. There were no cases of diabetes mellitus, Rh incompatibility or post-term pregnancies amongst the cases or controls, as these conditions are known to result in raised NRBC counts [16]. Nucleated red blood cells are seen in fetal circulation quite commonly although the numbers are variable. Mohanty A, et al. in 1995 found the mean numbers of NRBCs per 100WBCs + S.D non asphyxiated babies was 3.4 ± 3.0 with a range of 0-12. In asphyxiated babies, it was 34.5 ± 68.3 with a range of 1-451 [17]. Apgar V., et al. in 1997 found that in term singleton neonates the mean NRBC count was 8.55 per 100 WBCs with a standard deviation of 10.27 [18]. Sarnat H, et al. found it to be 7.56 ±3.85 in normal fetuses. The counts in acute asphyxia group were 11.18 ± 4.92 and 24.43± 20.05 in chronic asphyxia group. In our study the mean NRBC count ± S.D. in controls was found to be 12.33±5.5 land in asphyxiated neonates it was 25.65±10.14 with a range of 11-58 [19]. The mean NRBC count in the thick MASF group was 27.41±10.13 compared to the count of 13.53±6.37 in the clear and thin MASF group. Ferber et al in 2003, studied the relationship between abnormal FHR patterns and NRBC counts was 9 in cases with abnormal FHR patterns compared to 5 in controls. Out of the various abnormalities in FHR patterns the presence of declarations correlated best with raised NRBC counts but the stepwise regression analysis did not confirm this finding [20]. They concluded that this might be due to the high false positive rate of FHR patterns in predicting the perinatal outcome. Our study does not show a statistically significant correlation between NRBC counts and abnormal FHR patterns (P 0.519) yet the mean NRBC counts were higher for the non-reassuring FHR group i.e. 24.89±9.49 compared to 14.16±8.78 in the reassuring FHR group. NRBC counts when analyzed in relation to Apgar scores, were found to be inversely proportional to Apgar scores at both 1 and 5 minutes. 1-minute Apgar scores of 0-3 were significantly associated with raised NRBC counts than were Apgar scores of 7-10. Spencer et al found that NRBC count was a better predictor of acidosis than Apgar scores. The mean NRBC count in neonates with a 5-minute Apgar less than or equal to 6 was 26.23 ± 10.01 whereas in those with Apgar more than 6 was 13.04±6.42.In 1995 [21]. The mean NRBC counts in the neonates that were shifted to NICU was 29.80±10.9 and 20.00±5.26 in those neonates that did not need NICU admission. 15 out of 52 study group cases had significant neonatal morbidity in the form of severe birth asphyxia, seizures or hypoxic encephalopathy and 3 neonates expired [22]. Addock LM, et al. went on to suggest that the clearance of the NRBCs from the circulation could be of help in prognosticating the outcome of these asphyxiated neonates. The finding needs to be studied in detail in order to be able to assess the significant rate of clearance of NRBCs [23].

Conclusion

The Mean Nucleated RBC count in neonates with cord blood ph of 7.1 was twice that in

normal neonates who had cord blood ph.7.1. Neonates who showed thick msaf had higher mean NRBC count than those with clear and thin MSAF and the difference was statistically significant. Neonates who showed non-reassuring FHR had higher mean NRBC than with reassuring FHR and difference was not statistically significant. Neonates with low Apgar scores showed statistically significant raised NRBC count. NRBC count is a simple bedside test that can be used to diagnose fetal asphyxia.

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