

Comparative Study of Cord Blood Nucleated Red Blood Cell Count (NRBC) in Asphyxiated and Nonasphyxiated Babies at Birth in Tertiary Health Centre

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ABSTRACT

Introduction: Perinatal asphyxia is a major cause of neurological morbidity and mortality among survivors and is a complication that occurs between 5-10% of deliveries. The term asphyxia should be exclusively used to indicate those babies who have metabolic acidosis and hypoxia at birth.

Aim: The aim of study is to analyse whether the increase in NRBC in asphyxia is significant or not as compared to the nonasphyxiated group.

Material and Methods: This was a prospective comparative study in Department of OBG in tertiary care healthcentre. Cord blood samples were collected from 100 newborns with asphyxia at birth and equal number of normal appropriately

matched control to study NRBC per 100 WBC in Cord blood sample. The study was done during the period of December 2013 to November 2015 over 200 newborns. The statistical analysis was done using SPSS software version 16 and Chi-square test.

Results: This shows that mean count of NRBC/100 WBC in the study was 25.59 ± 7.19 (SD). Mean count in the control group was 12.69 ± 5.29 (SD). The difference was statistically significant ($p < 0.001$).

Conclusion: NRBC count can be useful for the evaluation of perinatal asphyxia, where facilities for pH Sampling are not available and can serve as a liable, inexpensive and easily available marker of perinatal asphyxia.

Keywords: Fetal asphyxia, Normoblast, Umbilical cord blood, White blood cell count

INTRODUCTION

Perinatal asphyxia should be exclusively used to indicate those infants who have metabolic acidosis and hypoxia at birth. There are various parameters described in literature to diagnose perinatal asphyxia like umbilical cord blood pH estimation, meconium stained amniotic fluid, Apgar score and fetal heart rate changes. Interest to work in this field was motivated by the fact, that in developing countries, there are inadequate facilities at the peripheral health centers to measure cord blood or fetal scalp pH, which best confirms the acidosis in cord blood. The correlation between clinical markers and fetal acidosis is not so much satisfactory according to previous studies, so clinical markers alone cannot be used as a marker of fetal asphyxia. In these situations NRBC/100 WBC count seems to be very feasible and practically possible at all levels of health care provision. The study aims to analyse the role of NRBC as a marker of fetal asphyxia. This study will help us in management planning as well as prognosticating the future outcome of the baby in aspect of hematopoietic response to hypoxia in utero NRBC per 100 WBC count is a good predictor of perinatal asphyxia [1,2].

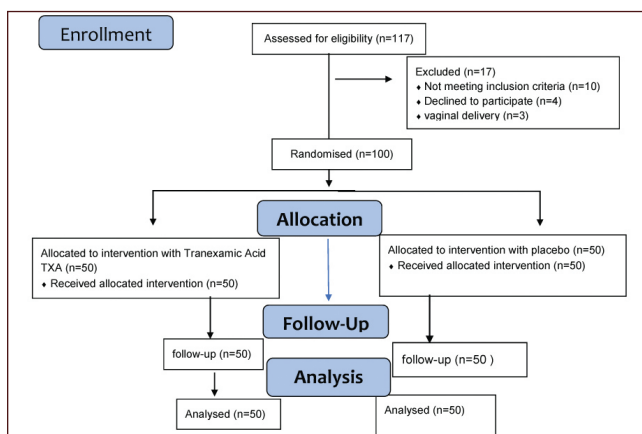
MATERIALS AND METHODS

This was a comparative study of cord blood NRBC count in asphyxiated and nonasphyxiated baby in a tertiary level health care center conducted between December 2013 to November 2015. Cord blood were collected from 100 newborn with asphyxia at birth and equal no of normal appropriately matched control to study NRBC per 100 WBC in cord blood sample. The sample size was calculated based on prevalence of neonatal hypoxia and odds ratio [3]. Ethical clearance number of the study was PMC/2013/2226. Informed consent was obtained for every patient. Patients in both case and control groups were selected from labour room patients. These were the patients who were having singleton pregnancy and were in labour between 37 to 42 weeks of gestation. All patients who had associated with acute and chronic hypoxia were excluded from this study. These factors may influence hematopoiesis so their presence may give false positive results [4].

Inclusion criteria: presence of >2 of following criteria:

- Thick meconium stained amniotic fluid
- Nonreassuring fetal heart rate pattern
- Low Apgar score ≤ 6 at 5 minutes of birth (followed up in NICU)

From all the subjects, cord bloods were collected immediately after clamping and cutting the umbilical cord. Sample was taken in an EDTA (Ethylene diamine tetra acetic acid) coated bottle for the purpose of making blood smear. For making smear, two glass slides were taken, a drop of blood was smeared over the slide and it was fixed with Giemsa solution. Prepared slide was examined under high power of microscope and number of NRBC was counted against the number of WBC until 100 WBC were counted [Table/Fig-1].



[Table/Fig-1]: Showing late NRBCs at upper left and lymphocyte at lower right.

STATISTICAL ANALYSIS

Statistical analysis was done using software Statistical Package for Social Science (SPSS) version 16 and chi-square test.

RESULTS

The study included 100 patients in study (asphyxiated baby) and 100 patients in control group (nonasphyxiated) for this analysis. Mean count of NRBC/100 WBC in the study group was 25.59 ± 7.19 (SD). Mean count in control group was 12.69 ± 5.29 (SD). The difference was statistically significant ($p < 0.001$). Majority of the asphyxiated babies (65%) had NRBC/100WBC count in the range of 20-29, whereas the maximum number of patients in the control group i.e., 61% had NRBC count in the range of 10-19. None of the asphyxiated babies had a normal NRBC count (normal NRBC count/100 WBC is rarely more than 10 at term). It was also observed that as the range of NRBC count increased the number of nonasphyxiated babies decreased. [Table/Fig-2] shows NRBC count in both asphyxiated and nonasphyxiated babies.

Out of 100 patients in the study group, 78 had thick meconium stained liquor. Rest 22 and all 100 patients in the control group had clear/thin meconium stained AF. Mean NRBC count/100 WBC in the thick MSAF group was more strongly associated

with fetal asphyxia than in the clear/thin MSAF. A total of 85 patients out of 100 in the study group, and 7 patients from the control group had non-reassuring fetal heart rate pattern during labour. Among the 100 patients in the study group 88 had Apgar score ≤ 6 and the rest 12 patients and 100 patients of control group had Apgar score > 6 . The p-value for all the clinical surrogate markers of asphyxia was less than 0.001 making them significant. [Table/Fig-3] shows correlation of NRBC count with clinical surrogate markers of fetal asphyxia.

NRBC /100 WBC	No. of Pts in study group	No. of Pts in control group
0-9	0	28
10-19	13	61
20-29	65	9
30-39	16	2
40-49	6	0
Mean NRBC/100 WBC	25.59 SD-7.19	12.69 SD-5.29

[Table/Fig-2]: NRBC Count/ 100 WBC (P <0.001).

Sr. no.	Clinical markers	No. of patients	Means NRBC/100 WBC \pm SD	p-value
1.	Amniotic fluid			
	Thick MSAF	78	27.28 \pm 6.69 (SD)	$p < 0.001$
	Clear/Thin MSAF	122	14.16 \pm 6.04 (SD)	
2.	FHR pattern			
	Nonreassuring	92	24.45 \pm 4.72 (SD)	$p < 0.001$
	Reassuring	108	14.71 \pm 7.73 (SD)	
3.	Apgar Score at 5 minutes			
	≤ 6	88	26.77 \pm 6.47 (SD)	$p < 0.001$
	> 6	112	13.39 \pm 5.69 (SD)	

[Table/Fig-3]: Correlation of NRBC count with clinical surrogate markers of fetal asphyxia

DISCUSSION

In the present study, NRBC count per 100 WBC in the study group (i.e., cord blood pH ≤ 7.1) ranged between 11 and 47 and in the control group (i.e., cord blood pH > 7.1) it ranged between 4 and 30. The maximum number of patients in the study group i.e., 65% had NRBC count in the range of 20-29, whereas maximum number of patients in the control group i.e., 61% had NRBC count in the range of 10-19. The mean NRBC/100 WBC in the study group was 25.59 ± 7.19 (SD) and the mean count in the control group was 12.69 ± 5.29 (SD). The difference was statistically significant ($p < 0.001$). Previous studies also showed that in fetal hypoxia, NRBC count was quite common though its number varied. NRBC count is an index of perinatal brain damage and helps in identifying perinatal hypoxia and in predicting neurodevelopmental outcome [5]. It not only reflects an adaptive response of infants to perinatal distress but also is predictor of an increased risk of brain damage. There

is a significant association between NRBC and umbilical cord pH ($P < 0.0001$) [2,6]. Phelan JP et al., found the NRBC count in normal nonasphyxiated neonates to range from 0-12 [7]. Spencer MK et al., summarised that in comparison to MSAF, Nonreassuring FHR, low Apgar scores and fetal erythropoietin levels NRBC count is a superior marker of fetal metabolic acidosis [8]. In this study mean NRBC count was higher in MSAF than in clear liquor and which was statistically significant. Saracoglu et al., found the mean NRBC count/100 WBC count in the acute distress and the chronic distress group as compared to the controls was statistically significant ($p < 0.05$) [9].

The results of the study by Ghosh B et al., were also consistent with our results as, the mean NRBC/100 WBC in the study group was 16.5 ± 6.4 (SD) and in the control group it was 8.6 ± 7.01 (SD) [10]. The difference was statistically significant ($p < 0.001$). In the study reported by Ferber A et al., in 2000, the median NRBC count was higher in cases with nonreassuring FHR pattern compared to nonasphyxiated baby. In our study, the mean count was higher in nonreassuring group and there was statistically significant correlation between NRBC count and abnormal FHR pattern [11]. McCarthy J et al., found that uncomplicated labour did not affect NRBC level [12]. The mean NRBC count in uncomplicated labour at term was 8.4 ± 8.7 (SD). In the study by Papa D et al., of JIPMER, the mean NRBC count in the study group was 25.65 ± 10.14 (SD) [13]. Their results were also consistent with our results. In the study by Tomar G et al., mean NRBC/100 WBC for the control (nonasphyxiated) and for the case (asphyxiated) group was statistically significant and consistent with results of this study [14].

Correlation of NRBC with amniotic fluid status

Meconium has long been suspected as a marker of fetal hypoxia. It is also related to the gestational age. Elevated erythropoietin level has been associated with the presence of meconium. Fetal hypoxia stimulates fetal evacuation of meconium, but most of the 5-20% of infants born with meconium stained amniotic fluid, do not develop respiratory symptoms.

In this study, out of 200 patients (including both the study and the control group) 78 had thick meconium stained liquor and in the rest liquor was either clear or thin meconium stained. Patients with thick MSAF were taken in the group with asphyxiated baby and the thin MSAF and clear AF were taken in the other group i.e., nonasphyxiated baby. NRBC count/100 WBC in the cord blood sample was compared between the two groups. NRBC count/100 WBC in the thick MSAF group was 27.28 ± 6.69 and the count in clear/thin MSAF was 14.16 ± 6.04 . The p-value was < 0.001 . Thus, the difference in the NRBC count between the two groups was statistically significant. This result was consistent with the previous study results.

In the study by Papa D et al., of JIPMER, India, the mean NRBC count/100 WBC in the thick MSAF group was 27.41 ± 10.13 (SD)

and in the clear/thin MSAF group, was 13.53 ± 6.37 (SD) p-value was 0.021 (< 0.05) which was statistically significant [13].

In the study by Buonocore G et al., in 1991-95, the mean NRBC count was increased significantly in the babies delivered by patients with MSAF [15]. Lundberg H et al., in 1996-97 studied NRBC in the neonates with acidemia. In their study presence of meconium was associated with higher NRBC count i.e., 12 ± 26.4 . The p-value was 0.020 (< 0.05) which was statistically significant [16]. Dollberg S et al., studied NRBC count in meconium aspiration syndrome. They found NRBC count higher in neonates with symptomatic meconium aspiration syndrome ($0.007 \times 10^9/l$) as compared to asymptomatic meconium aspiration group ($0.004 \times 10^9/l$). In the control group, mean NRBC count was $0.003 \times 10^9/l$. The p-value was < 0.02 which means the difference was statistically significant [17]. Spencer MK et al., 1997-98, in their study found meconium stained liquor to be having 34% positive predictive value in diagnosing fetal metabolic acidosis [8]. In the study by Ghosh B et al., of AIIMS, a statistically significant correlation was found between NRBC count and MSAF [10].

Correlation of NRBC count with FHR pattern

The original goal of fetal heart rate monitoring was to improve perinatal outcome by early recognition of fetal hypoxia. Elevated umbilical cord NRBC and abnormal FHR pattern, both reflect fetal hypoxia. However, the relationship between these variables has received very little attention. In this study, NRBC count was correlated with the FHR pattern. Out of the 200 patients, 92 had nonreassuring FHR pattern during labour, and 108 patients had reassuring FHR pattern.

Mean NRBC count/100 WBC in the non-reassuring group was 24.45 ± 4.72 (SD) where as in the reassuring group, it was 14.71 ± 7.73 (SD). The difference between the two readings was statistically significant ($P < 0.001$). Out of 92 patients in non-reassuring group, 7 patients were those, whose cord blood pH was > 7.1 and in the rest 85 pts, cord blood pH was ≤ 7.1 . NRBC count was higher in the nonreassuring group and this was statistically significant.

In the study by Buonocore G et al., in 1991-95, it was found that NRBC was higher in neonates with abnormal FHR pattern in term as well as preterm babies ($P < 0.001$) [15].

Phelan JP et al., in 1996 grouped the infants on the basis of FHR pattern and concluded that the closer the birth was to the asphyxia event, the lower was the number of NRBC [7]. Spencer MK et al., in 1997-98 in their study found that abnormal FHR pattern had 34% positive predictive value [8].

In the study by Ferber A et al., in 2000, the mean cord blood NRBC count/100 WBC in subjects with abnormal FHR pattern was 9 and in those with normal FHR pattern it was 5. The difference was statistically significant ($P < 0.0001$) [11]. This was consistent with the results of the present study. They also

emphasised that the presence of FHR acceleration is the only abnormality that independently predicts elevated NRBC count at birth.

Papa D et al., in 2002 found that the mean NRBC /100 WBC in the nonreassuring group was 24.89 ± 9.49 and in the reassuring group was 14.16 ± 8.78 ($p=0.519$). The NRBC count was definitively higher in the abnormal FHR pattern group but, the difference was not statistically significant according to their study [13].

Correlation of NRBC with Apgar score

In this study, out of 200 patients, 88 patients delivered babies with Apgar score ≤ 6 at 5 minutes of birth. All these patients were in the study group i.e., they were having birth asphyxia and the umbilical cord arterial pH was ≤ 7.1 . A total of 12 patients in the study group had Apgar score >6 and all 100 patients in the control group also had Apgar score >6 .

Mean NRBC count/100 WBC in the group with Apgar score ≤ 6 was 26.77 ± 6.47 (SD) and in the group with Apgar score >6 , the mean NRBC count /100 WBC was 13.39 ± 5.69 (SD). The p-value was <0.001 . So the difference was statistically significant. All of the 56 neonates who were admitted to NICU, 54 had Apgar score ≤ 6 and only 2 had Apgar score of 7.

Spencer MK et al., in 1997-98 found that the positive predictive value of Apgar score in fetal metabolic acidosis was 75%, which was higher than the other clinical parameters [8]. In the study by Lundberg H et al., in 1996-97, it was found that NRBC count had inverse relationship with Apgar score at both 1 and 5 minutes and NRBC was significantly elevated in cord blood in babies with low Apgar score at 1 and 5 minutes [16]. In the study by Ghosh B et al., in 1999-2001, the mean Apgar score in the asphyxiated babies was 3 and in the nonasphyxiated babies, it was 9. There was statistically significant negative correlation between NRBC and Apgar score [10].

In the study by Papa D et al., of JIPMER in 2002-04, NRBC count was higher in the newborns with Apgar score ≤ 6 at 5 minutes of birth, mean value of NRBC /100 WBC in low Apgar score group was 26.23 ± 10.01 (SD) Whereas in the babies having Apgar score >6 at 5 minutes of birth, the mean NRBC/100 WBC was 13.04 ± 6.42 (SD) [13]. Nucleated red blood cell count at birth is a useful predictor of severity and short-term outcome of perinatal asphyxia [18] some studies show serum levels of IL-6 increased in neonates with asphyxia, and this was most pronounced in neonates with adverse outcomes [19].

Thus, we can see that in the present study and many previous studies NRBC count in cord blood shows negative correlation with Apgar score. The study was limited only to the singleton pregnancy without any comorbidities so the results cannot be generalised over the entire population.

CONCLUSION(S)

Perinatal asphyxia is diagnosed by several criteria; the two important ones are cardio-respiratory and neurological depression. From this study, it can be concluded that NRBC count correlates well with fetal acidosis in asphyxiated neonates and this is a simple bed side test, the sample being obtained non invasively from an otherwise discarded specimen and analysed by personnel or equipment easily available in most hospital laboratories. This test has a useful role to play in situations (including litigations) where the retrospective identification of acidosis is required. However, confounding factors that may also increase NRBC have to be excluded through more extensive studies. In resource poor countries, it may be impossible to assess fetal or neonatal acidemia by cord blood pH. Hence, NRBC count can be a useful tool for the evaluation of perinatal asphyxia, where facilities for pH sampling are not available and can serve as a liable, inexpensive and easily available marker of perinatal asphyxia. The study can be more precise if acute and chronic confounding conditions are ruled out as they may cause spurious rise in NRBC count.

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