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Original Research Article

Correlation of Elevated Cord Blood Nucleated RBC Count (nRBC) with Common Antenatal Risk Factors and Outcome in Term and Preterm Neonates

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Abstract

Introduction: Nucleated red blood cells (nRBCs) in umbilical cord blood are recognized as markers of fetal hypoxia, inflammation, and intrauterine stress. Elevated cord blood nRBC counts have been associated with adverse perinatal outcomes, including low APGAR scores, neonatal intensive care unit (NICU) admission, respiratory distress, sepsis, and mortality.

Aim: This study aimed to evaluate the association of cord blood nRBC counts with antenatal risk factors and neonatal outcomes.

Materials and Methods: A prospective observational study was conducted from July 2025 to January 2026. A total of 130 mother -neonate's pairs were included. Cord blood samples were collected immediately after delivery and analyzed for complete blood count and nRBC estimation using an automated hematology analyzer and peripheral smear examination. Maternal, intrapartum, and neonatal variables were recorded. Statistical analysis was performed using SPSS version 45, with $p<0.05$ considered significant.

Results: Among 130 neonates, 55 (42.3%) had elevated nRBC counts (>10/100 WBC). Elevated nRBC counts were significantly associated with pregnancy-induced hypertension (p=0.0074), maternal anemia (p=0.0134), antepartum hemorrhage (p=0.0122), thick meconium-stained liquor (p=0.0074), and multiple antenatal risk factors (p=0.0203). Neonates with elevated nRBC counts had higher rates of respiratory distress syndrome, hypoxic-ischemic encephalopathy, meconium aspiration syndrome, abnormal APGAR scores, NICU admission, prolonged NICU stay (Spearman's rho=0.83), and mortality. Out of 10 neonatal deaths, 9(90%) had elevated nRBC counts (p=0.0019).

Conclusion: Umbilical cord blood nRBC count is a simple, inexpensive, and reliable marker of antenatal fetal stress. Elevated nRBC levels are strongly associated with neonatal morbidity, prolonged NICU stay, and mortality, making them a valuable adjunct for early risk stratification and prediction of neonatal outcomes.

Keywords: Neonate, Reticulocyte Count, nRBC, Antenatal risk factors.

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Introduction

Nucleated red blood cels (nRBCs) are immature erythroid precursors that are normally present in the fetal circulation and rapidly decline after birth. Their presence in cord blood has been increasingly recognized as an important haematological marker of intrauterine stress, particularly in conditions associated with chronic or intermittent fetal hypoxia and inflammation. [1] In the presence of adverse intrauterine environments such as pregnancy- induced hypertension, placental insufficiency, maternal diabetes, or fetal distress, there is enhanced erythropoiesis leading to elevated nRBC counts in cord blood. [2,3] Several studies have demonstrated that elevated cord blood nRBC counts are associated with poor perinatal outcomes, including low APGAR scores, need for resuscitation, neonatal intensive care unit(NICU) admission, and increased risk of morbidity such as respiratory distress syndrome, sepsis, and hypoxic ischemic encephalopathy. [4,5] Despite its clinical relevance, the utility of cord blood nRBC count as a simple, early, and cost-effective marker for predicting neonatal outcome remains underutilized in routine clinical practice. Moreover, the relationship between nRBC levels and various antenatal risk factors has not been uniformly established across different populations.

Material and Methods

This study was a hospital based prospective observational study, conducted in the department of Pediatrics, Obstetrics and Gynecology, study was approved by institutional ethical committee. Written informed consent was obtained in the local language. Participation was voluntary; withdrawal from the study was allowed anytime. Confidentiality was maintained throughout the study.

This study was carried out over a period of from July 2025 to January 2026 and included term and preterm neonates born at gestational age 28 weeks–42 weeks delivered in the institute. Newborns with Extreme prematurity (<28 weeks),birth weight <1000 gm , congenital malformations, Rh negative mother , ABO incompatibility and those delivered outside institute were excluded from the study. Informed consent was obtained from antenatal mothers registered during the study period. 2ml disposable plastic syringe and needle was kept ready after delivery, the umbilical cord was double clamped and cord blood was collected from the placental side of the umbilical cord in EDTA vacutainer meticulously and under strict aseptic precautions, taking care that wharton's jelly is not mixed with the blood. Cord blood was sent for complete blood count (CBC) and nRBC estimation, using an automated haematology analyser (Yumizen Horiba 2500).

A peripheral smear prepared from the cord blood sample was stained using Leishman stain, and nRBCs were counted manually as the number of nucleated RBCs per 100 WBCs. nRBC count was expressed as Number of nucleated red blood cels per 100 white blood cels (nRBCs/100 WBCs). Maternal data including age, Anaemia, Gestational diabetes melitus, Pregnancy-induced hypertension, and foetal wel-being were obtained from case records.

Intrapartum/natal risk factors such as prolonged second stage of labour, mode of delivery, presence of antepartum haemorrhage, premature rupture of membrane > 24 hr, meconium-stained amniotic fluid, and need for resuscitation were noted. Neonates were examined soon after birth and data including birth weight, Gestational age and APGAR scores were recorded. Neonatal outcomes including morbidity (neonatal problems, NICU admission, and duration of NICU stay and mortality were documented.

The sample size was calculated to estimate the proportion of neonates with elevated cord blood nRBC counts using the formula for a single proportion:

$$n = Z^2 \times P \times P\ (1-p)\ d^2$$

Thus, the minimum required sample size is approximately 92 neonates. Considering feasibility and to improve study power, 130 neonates were finally included.

Data collection variables included Antenatal variables: Maternal Age, Anaemia, Gestational Diabetes Melitus, Pregnancy-Induced Hypertension, and Foetal Wel-Being. Natal variables: Mode of Delivery, Prolonged Second Stage of Labour, Meconium-Stained Amniotic Fluid, Resuscitation at Birth. Neonatal variables : Gestational Age, Birth Weight, APGAR Scores, nRBC Count, NICU Admission and Duration, Morbidity, Mortality.

Data were entered into Microsoft Excel and analyzed using SPSS version 45. Continuous variables were expressed as mean ±standard deviation. Comparison between groups was done using Student's t-test for continuous variables and Chi-square test or Fisher's exact test for categorical variables. Spearman correlation was used to assess relationship between nRBC count and duration of NICU stay. A p value of 0.05 was considered as statistically significant.

Results

Out of the 130 neonates included in the study, 71 (54.6%) were females and 59 (45.4%) were males. Thus, female neonates were slightly more than male neonates. Out of the total 130 neonates, 50 (38.5%) required NICU admission, while 80 (61.5%) did not require NICU care, duration of NICU stay ranged from 0 to 25 days.

The mean duration was 3.62 ± 5.85 days, while the median duration was 0 days. The age of the mothers ranged from 22 to 36 years. The mean maternal age was 27.7 ± 3.36 years, while the median maternal age was 28years.

Most mothers were in the late twenties, indicating that the majority belonged to the normal reproductive age group. 50 mothers (38.5%) were primigravida/primiparous, whereas 80 mothers (61.5%) were multigravida/ multiparous.

The commonest mode of delivery in this study was normal vaginal delivery, which was seen in 73 cases (56.2%) and caesarean section in 52 cases (40.0%) and instrumental delivery in 5 cases (3.8%).

Among the 52 caesarean sections, emergency caesarean section for foetal distress was the commonest indication and was seen in 21 cases (40.4%). Antenatal risk factors were present in a substantial proportion of mothers.

Out of the total 130 cases, 85 (65.4%) had one or more antenatal risk factors and 45 (34.6%) had no antenatal risk factor. The common antenatal risk factors included pregnancy-induced hypertension,

meconium-stained liquor, gestational diabetes, and maternal anaemia, which is depicted in table-1.

Table 1: Distribution of Antenatal risk factors in 130 mothers

Antenatal Risk factors (Variable)	YES	NO
Pregnancy-induced hypertension (PIH)	33 (25.4%)	97 (74.6%)
Gestational diabetes mellitus (GDM)	27 (20.8%)	103 (79.2%)
Maternal anaemia	25 (19.2%)	105 (80.8%)
Oligohydramnios	17 (13.1%)	113 (86.9%)
PROM (Premature rupture of membrane)	19 (14.6%)	111 (85.4%)
Thick meconium	15 (11.5%)	115 (88.5%)
Thin meconium	12 (9.2%)	118 (90.8%)
Antepartum haemorrhage (APH)	5 (3.8%)	125 (96.2%)
Prolonged 2nd stage of labour	3 (2.3%)	127 (97.7%)
PROM>24hours	2 (1.5%)	128 (98.5%)

Table 2: Distribution of Non-Stress Test (NST) findings

Category	N	Percentage
Reactive	93	71.5
Non-Reactive	37	28.5

NST findings were reactive in 93 cases (71.5%) and non-reactive in 37 cases (28.5%).

Therefore, reactive NST constituted the majority, while nearly one-third of mothers had non-reactive NST findings. This suggests that most pregnancies had reassuring antepartum foetal surveillance findings. However, the presence of non-reactive NST in a considerable proportion also indicates that a significant number of foetus had antenatal evidence of possible compromise. Out of the 130 neonates, 48 (36.9%) required resuscitation in the form of tactile stimulation, positive pressure ventilation or intubation at birth.

Thus, the majority of neonates were born without the need for active resuscitation. Average gestational age of the study population70% was close to full term (37.92±2.21) mean birth weight was (2.90 ± 0.60).

Table 3: Types of complications, in Newborn (n=130)

Category		Number (%)
No Complications		81(62.3%)
Complications		49(37.7%)
Complications(Variables)	Respiratory Distress Syndrome (RDS)	23(17.7%)
	Meconium Aspiration Syndrome (MAS)	10 (7.7%)
	Sepsis	9(6.9%)
	Transient Tachypnoea	6(4.6%)
	Hypoxemic Ischemic Encephalopathy	3(2.3%)
	Intraventricular Hemorrhage	2(1.5%)
	Hypoglycemia	1(0.8%)
	≥ 2 Risk factors	39(30%)
	≥3 Risk factors	30(23.1%)

Some of the above variables were common and present in two or more than two risk factors.

Table 4: Neonatal hematological parameters (Mean, Median and Range)

Neonatal Parameter	Mean ± SD	Median	Range
Hemoglobin (gm/dl)	16.77±1.63	16.4	12.5–20.1
Total Leukocyte Count	16,644.62 ± 4,509.47	14,500	11,800–29,000
nRBC (Nucleated Red Blood Cells) / 100 WBC	15.08 ± 16.23	6	2-65

Elevated nucleated RBCs (>10/100 WBC) were found in 55 (42.3%) while 75 (57.7%) newborns did not have elevated nucleated RBCs.

Table 5: Correlation of Mode of delivery with elevated nRBC (cutoff >10) (n=130)

Mode of delivery	Elevated nRBC (%)	Non-elevated nRBC (%)	Total (110)
Caesarean Section	42 (80.8)	10 (19.2)	52
Instrumental Delivery	5 (100.0)	0 (0.0)	5
Normal Vaginal Delivery	8 (11.0)	65 (89.0)	73

Elevated nRBC count was seen in 42 of 52 neonates (80.8%) delivered by caesarean section and in all 5 instrumental deliveries (100.0%), whereas only 8 of 73 neonates (11.0%) delivered by normal vaginal delivery had elevated nRBC count. This suggests that elevated cord blood nRBC count was much more frequent in neonates delivered by operative methods.

Table 6: Correlation of Antenatal risk factors and nRBC

Antenatal risk factors in pregnant mothers	No. of elevated n RBC (%)	No. of non-elevated n RBC (%)	Median nRBC /100 WBCs	Range	P value
Pregnancy-induced hypertension (PIH)	21(38.18)	12 (16.00)	26.0	18–60	0.0074
Gestational diabetes mellitus (GDM)	10(18.18)	17 (22.67)	12.5	11–48	0.6626
Maternal anaemia	5(9.09)	20 (26.67)	28.0	23–42	0.0134
Oligohydramnios	6(10.1)	11 (14.67)	26.0	12–60	0.6059
PROM	8(14.55)	11 (14.67)	21.5	13–55	1.0000
PROM >24 hours	2 (3.64)	0 (0.00)	14.0	13–15	0.1771
Antepartum haemorrhage (APH)	5(9.09)	0 (0.00)	42.0	28–60	0.0122
Thick meconium	14(25.45%)	12 (16.00)	50.0	31–65	0.0074
Thin meconium	6(10.91)	17 (22.67)	13.0	11–14	0.6626
Prolonged 2nd stage of labour	3 (5.45)	20 (26.67)	42.0	42–48	0.0134
≥2 risk factors	16(29.09)	23 (30.67)	33.0	21–60	1.0000
≥3 risk factors	7(12.73)	23(30.67)	42.0	28–60	0.0203
No risk factors	0(0)	45(60)	—	—	<0.0001

Higher nRBC count was seen significantly more often in babies whose mothers had pregnancy-induced hypertension, antepartum hemorrhage, and thick meconium.

On the other hand, maternal anemia and the presence of three or more antenatal risk factors were seen more often in the non-elevated nRBC group. The absence of antenatal risk factors was significantly associated with non-elevated nRBC counts ($p < 0.0001$), which is an expected finding as these neonates were less likely to be exposed to intrauterine stress.

Multiple antenatal risk factors could be present in the same mother, therefore, risk factor categories are not mutually exclusive and the sum of the individual frequencies exceeds, total number of neonates in each group. The categories ≥ 2 risk factors and ≥ 3 risk factors represents overlapping subsets of mothers and was not added to the individual risk factor frequencies.

Table 7: Correlation of nRBCs and Neonatal Complications

Neonatal complications	No.of elevated nRBC Cases	No.of non elevated nRBC Cases	Median nRBC/100 WBCs	Range	P value
None	9	1(1.33)	12.0	11–42	<0.0001
Respiratory Distress Syndrome	22(40)	0	23.5	13–45	0.0003
Sepsis	9(16.36)	0	33.0	13–60	0.1771
IVH (Intraventricular haemorrhage)	2(3.64)	0	41.5	38–45	0.0733
HIE (Hypoxic ischemic encephalopathy)	3(5.45)	0	60.0	31–65	0.0001
MAS(Meconium Aspiration Syndrome)	10(18.18)	2(2.67)	50.0	42–60	0.2411
Transient Tachypnea	4(7.27)	0	14.0	11–14	0.4231
Hypoglycemia	1(1.82)	0	21.0	21–21	<0.0001
≥2 risk factors	5	0	-	-	0.0122

The frequencies of individual neonatal complications do not add up to the total number of neonate in each nRBC group because some neonates had more than one complication and were counted in multiple categories.

In addition, a proportion of neonates had no complications. Hence the complication frequencies are not mutually exclusive, and the sum of individual complication counts does not equal the total number of neonates in the elevated and non-elevated nRBC groups.

Among neonates with abnormal APGAR score at 1 minute (cut off <7), 29 of 30 babies (96.67%) had elevated nRBC count. In contrast, among neonates with normal APGAR score at 1 minute, only 26 of 100 babies (26.0%) had elevated nRBC count. This shows a strong association between elevated nRBC count and poor immediate condition at birth.

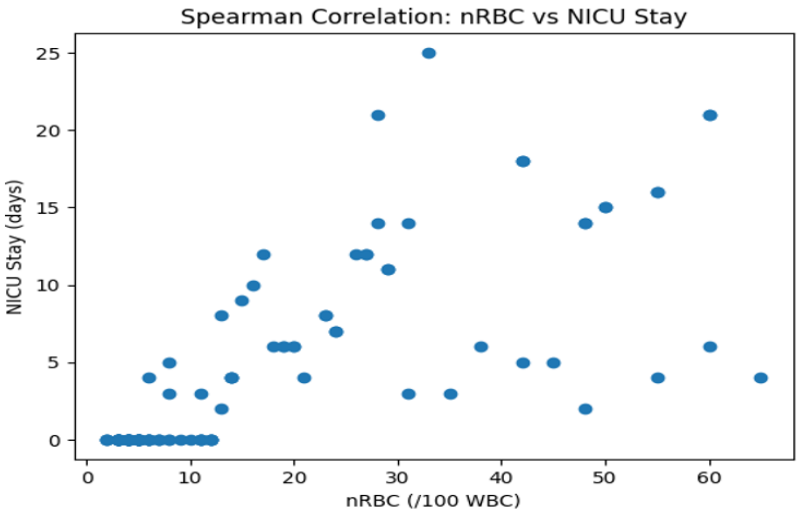

Figure 1: Correlation of elevated nRBC with duration of NICU stay

A strong positive correlation was observed between cord blood nRBC count and duration of NICU stay (Spearman's rho = 0.83), indicating that higher nRBC values were associated with prolonged NICU hospitalization (as above). Out of 130 neonates, 120 neonates (92.3%) were discharged, while 10 neonates (7.7%) expired.

Table 8: Correlation of nRBC with Morbidity, Mortality and Discharge

Variable	Category	Expired (n=10)	Discharged (n=120)	p-value
Cord Blood nRBC Status	Elevated	9 (90.0%)	46 (38.3%)	0.0019
	Non- elevated	1 (10.0%)	74 (61.7%)	
Antenatal Risk Factors	Present	10 (100%)	75 (62.5%)	0.0149
	Absent	0 (0%)	45 (37.5%)	
Neonatal Complications	Present	10 (100%)	39 (32.5%)	<0.0001
	Absent	0 (0%)	81 (67.5%)	

A statistically significant association was observed between elevated cord blood nRBC count and neonatal mortality (p = 0.0019). Similarly, the presence of antenatal risk factors (p = 0.0149) and neonatal complications (p < 0.0001) were significantly associated with mortality. Majority of expired neonates had elevated nRBC levels (90%), and all had associated antenatal risk factors and neonatal complications, highlighting a strong relationship between intrauterine stress, postnatal morbidity, and adverse outcome.

Discussion

In this study, a total of 130 neonates were included. A substantial proportion of mothers had one or more antenatal risk factors, with 85 out of 130 cases (65.4%) showing some antenatal risk, while 45 cases (34.6%) had no identified risk factor. Kil et al. [6] studied 112 very low birth weight infants and found an almost equal sex distribution, with 55 males (49.1%) and 57 females (50.9%) whereas, in the study, 71 (54.6%) were females and 59 (45.4%) were males, in this study. Darkhaneh et al. [7] observed a mean maternal age of 27.54 ± 4.66 years, with almost no difference between the preeclampsia group (27.48 ± 5.07 years) and the control group (27.56 ± 4.53 years) Zakerihamidi et al. [8] reported mean maternal age of 28.87 ± 6.30 years in the vaginal delivery group and 30.31 ± 6.73 years in the emergency caesarean section group in this study, maternal age ranged from 22 to 36 years, with a mean age of 27.70 ± 3.36 years and a median age of 28 years. Aali et al. [9] evaluated 50 pre-eclamptic women and 150 healthy pregnant women and found that, the mean cord blood nRBC count was 18.2 ± 31.8 per 100 WBC in the pre-eclamptic group, compared with 6.2 ± 8.1 per 100 WBC in controls. They also noted that low birth weight and intrauterine growth restriction had a significant relationship with abnormal nRBC counts. Hebbar et al. [10] studied 50 preeclamptic women and 50 healthy pregnant women and reported a mean cord blood nRBC count of 40.0 ± 85.1 in the preeclampsia group versus 5.9 ± 6.3 in controls, while the mean maternal nRBC count was 2.4 ± 9.0 versus 0.8 ± 1.5. Their study further showed that elevated cord blood nRBC counts were associated with IUGR, low birth weight, neonatal ICU admission, respiratory distress syndrome, and assisted ventilation. Dulay et al. [11] analysed 68 preterm singletons and found that neonates with early-onset neonatal sepsis (n = 19) had significantly higher absolute nRBC counts, with nRBC levels showing a direct correlation with cord blood interleukin-6, supporting an inflammatory pathway for nRBC rise even in the absence of

definite hypoxia. In this study, antenatal risk factors were present in 85 out of 130 mothers (65.4%), while 45 mothers (34.6%) had no antenatal risk factor. The common antenatal risk factors observed were pregnancy-induced hypertension, thick and thin meconium-stained liquor, gestational diabetes mellitus, maternal anaemia, oligohydramnios, PROM, APH, and various combined high-risk conditions.

Thus, nearly two-thirds of the mothers in this study had one or more antenatal risk factors. Hebbar et al. [10] studied 50 preeclamptic women and 50 healthy pregnant women and observed that among the 33 non-IUGR neonates of preeclamptic mothers, 8 (24.2%) required NICU admission. Darkhaneh et al [7]. Reported elevated nRBC counts in neonates of preeclamptic mothers, which were associated with low Apgar scores and increased NICU admission. Davari-Tanha et al [11]. Showed that nRBC counts were significantly higher in growth-restricted fetuses, reflecting chronic intrauterine hypoxia due to placental insufficiency. In addition, Dulay et al [12]. Highlighted that inflammatory mediators can independently increase nRBC production, even in the absence of hypoxia.

These studies support the concept that elevated nRBC count is a reliable marker of antenatal fetal stress. In the same subgroup, 4 neonates (12%) had respiratory distress syndrome and 4 (12%) required assisted ventilation, showing that higher nRBC counts were linked with clinically important neonatal morbidity. Hanlon-Lundberg and Kirby[13] evaluated 1,561 term neonates and found that elevated nRBC counts were significantly associated with NICU admission, along with foetal acidemia and meconium. Darkhaneh et al. [7] compared 50 neonates born to preeclamptic mothers with 150 neonates born to normotensive mothers and noted that higher neonatal complications, including greater need for NICU admission, in the preeclampsia group. In our study, 50 out of 130 neonates (38.5%) required NICU admission, while 80 neonates (61.5%) did not require NICU care. Thus, although most babies could be managed without intensive care, a considerable proportion still needed specialized monitoring and treatment. Hebbar et al.10 reported NICU admission in about one-fourth of the non- IUGR neonates of preeclamptic mothers, whereas in our study the proportion requiring NICU admission was higher (38.5%).Hanlon- Lundberg and Kirby [13] also support the clinical importance of this finding, because they showed that NICU admission tends to increase when foetal stress markers such as nRBC counts rise. Darkhaneh et al. [7] similarly pointed toward greater neonatal compromise in the preeclampsia group. In the present study, a higher proportion of neonates with adverse outcomes (mortality) were found to have elevated cord blood nRBC counts, as compared to those who were discharged. This suggests a strong association between elevated nRBC levels and poor neonatal outcome. Elevated nRBC count reflects increased erythropoietic activity secondary to intrauterine hypoxia or inflammation. Therefore, neonates with higher nRBC counts are more likely to have experienced significant antenatal or perinatal stress, which may predispose them to severe complications and increased risk of mortality. These findings are consistent with previous studies such as those by Minior et [14]. and Ferns et al [15], which demonstrated that elevated cord blood nRBC counts were significantly associated with increased neonatal morbidity and mortality.

Conclusion

Umbilical cord blood count (nRBC) is a simple, inexpensive and feasible hematological parameter, a useful supportive marker and a practical adjunct in assessing antenatal fetal distress in high risk pregnant mothers and hence in predicting early neonatal morbidity, mortality and neonatal outcome.

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