Original Research Article

Nucleated Red Blood Cells as a Prognostic Marker in Neonatal Intensive Care Unit

Abstract

Background: Nucleated red blood cells (NRBCs) are normally present in the circulation of the fetus during the intrauterine life but they disappear early in the first 10 postnatal days in healthy neonates.We aimedto assess the relations between NRBCs and the prognosis in neonatal intensive care unit (NICU).

Patients &Methods: This prospective cohort study was carried out on 120 neonates admitted to Tanta University NICU from January 2021 to January 2022. Full maternal history taking,detailed history of resuscitation andcomplete systemic examination were done. Threesamples for complete blood picture analysis & NRBCs count were taken in 1st 24 hours of admission, between 2nd -10th day, and after 10th day of admission.

Results: 32 cases out of 120 cases had NRBCs beyond normal range (>10/100WBCs) in the 1st 24 hours of admission;28 of them died (87.5 %). 19 cases had NRBCs beyond normal range between the 2ndand 10thday;17 of them died (89.5%).31 cases had detectable NRBCs after 10th day; NRBCs 30 of them died (96.8%).There was a statistically significant positive correlation between the presence of NRBCs count above normal range and the number of deceased cases(P = 0.001).

Conclusions: Nucleated red blood cells monitoring is a promising affordable marker that can predict poor prognosis in NICU.

Keywords: Nucleated Red Blood Cells; Prognostic Marker; Neonatal Intensive Care Unit.

Introduction

Nucleated red blood cells (NRBCs) are normally presentin the circulation of the fetus during the intrauterine life but they disappear early in the first 10 postnatal days in healthy neonates [1].The increased number of NRBCs has been considered a flag of intrauterine and early postnatal stress among neonates and were associated with increased risk of immediate post-natal complications.

Therefore, NRBCs can be a promising markers of severe illnessandbeusefulformonitoringcriticallyillinfantpatientsbeyondthefirstdays oflife.

The aim of this study was to assess the relation between the presence of NRBCs in circulating blood & the prognosis in NICU.

Patients and Methods

This prospective cohort study was carried out on 120 neonates who were admitted to NICU, Tanta University Hospital during one year from January 2021 to January 2022. The study was done after being approved from the institutional ethical committee, Tanta University (code:). Informed written consent was obtained from the parents of the patients' guardians.

Inclusion criteria: neonates admitted at NICU.

Exclusion criteria were neonates who were not subjected to CBC analysis on admission.

For all patients included in the study the following was done: Full maternal history taking including mode of delivery maternal medical problems or risk factors (e.g., maternal diabetes, anemia, smoking, urinary tract infection and bronchial asthma). Full maternal pregnancy history (e.g., pre-eclampsia, antepartum hemorrhage, prolonged delivery, abnormal presentation, gestational diabetes & placenta previa). Detailed history of

resuscitation, APGAR at 1st minute & 5thminutes, meconium staining of amniotic fluid & assessment of gestational age using New Ballard score [2].

Complete systemic examination. Complete blood picture collected at the first day of admission as 2ml of blood were collected in ethylene diamine tetra-acetic acid (EDTA) tube mixed thoroughly for complete blood picture analysis [3]. Another sample was collected between the next 2nd&10th day of admission with nucleated red blood cells counting with comparison with the previous sample, it was either increasing, decreasing, or newly appeared. One more sample was repeated after the 10th day of admission. Automatic blood cell counter (ERMA PCE-210N) was used for complete blood picture analysis. Then blood film was used for NRBCs counting [4]. The cases were followed up till they were discharged either alive or deceased.

Statistical analysis:

Using version 23.0 of the statistical program for social sciences, data were examined (SPSS Inc., Chicago, Illinois, USA). The quantitative information was reported as mean standard deviation (SD). The qualitative data were represented in terms of numbers and proportion. A one-way analysis of variance (ANOVA) is used to compare more than two means. Tukey's test was employed for multiple comparisons between various variables as a Post Hoc test. A relationship between the outcome of the neonates and nucleated red blood cells appearance was statistically analyzed using Chi-Square test (χ^2). Chi-Square test of independence is used to test whether the two categorical variables were related to each other and $P<0.05$ was adopted as the level of significance.

Results:

Demographic data of the studied caseswere shown in **Table 1**.

Among the 120 cases included in the studyin the 1st 24 hours: 49 (40.8%) cases had detectable level of NRBCS in the 1st24 hours of admission. 32 cases had NRBCs beyond

normal range (>10/100WBCs). This resembles:65.3% of 49 cases with detectable level of NRBCs.26.6 % of all 120 cases of the study.4 of them discharged alive (12.5% of 32 cases with NRBCs number beyond normal range). 28 of them deceased (87.5 % of 32 cases with NRBCs number beyond normal range). **Table 2,3**

Among the 120 cases between the 2nd and 10th day of admission: 52 (43.3%) cases had detectable level of NRBCS between the 2nd -10th days of admission.19 cases had NRBCs beyond normal range (>10/100WBCs). This resembles: 36.5% of 52 cases with detectable level of NRBCs.15.8% of all 120 cases of the study.3 cases with newly appeared high NRBCs beyond normal range (all died 100%) resembling (15.7% of 19 cases with NRBCs beyond normal range) & (2.5 % of all 120 study cases). 10 cases with increasing NRBCs (all died 100%) resembling (52.6% of 19 cases with NRBCs beyond normal range) (8.3% of all 120 of study cases). 6 cases with decreasing NRBCs in follow up. (31.5%of 19 cases with NRBCs beyond normal range) (5%of all cases). (2 of the 6 cases discharged alive (33.3%), 4 died (66.6 %).Relation between NRBCs presence between 2nd-10thday & neonatal outcome was shown in **Table 4**.

Among 120 cases (in follow up after 10th day): 31 cases with detectable NRBCs(25.8 % of all 120 cases). 14 cases with newly appeared NRBCs (all died 100%). Resembling 45.2% of 31 cases with detectable level of NRBCs.16 cases with increasing NRBCs (all died 100%).Resembling 51.6% of 31 cases with detectable level of NRBCs. One case with decreasing NRBCs (discharged good). Resembling 3.2% of 31 cases with detectable level of NRBCs.

According to the previous descriptive results Chi-squared test was used showing that there was a statistically significant relation between the presence of NRBCs count above normal range and the number of deceased cases. (P value 0.001). **Table 5**

Discussion

The present study was conducted to evaluate the role of NRBCs count in the prognosis of morbidity and mortality in critically ill neonates. Their results revealed that NRBCs could serve as prognostic marker in this population, especially in preterm neonates [5]. Inflammation, hypoxia, or massive hemorrhage seem responsible for the appearance of NRBCs in peripheral blood, as these situations increase erythropoietic pressure and result in failure of the spleen to remove these cells from the circulation. **Purtle et al.**demonstrated that the presence of NRBCs is associated with a significant increase in the odds of post-discharge hospital mortality in critically ill infants [6].

Schaer et al. found that NRBCs are not an independent risk factor for bad outcomes in pediatric intensive care; however, they mayhave a prognostic value in the first month of life, although their association with outcome is much less pronounced beyond the neonatal period[7].

Boskabadi et al. found that NRBCs count in neonates with birth asphyxia were significantly higher than healthy controls and associated with poorer short-term outcome.the study showed that NRBCs count could serve as a discriminator marker for early diagnosis of perinatal hypoxia with excellent performance in preterm neonates by using a cut-off value of ≥11.2%, with 80% sensitivity and 88.7% specificity [8].

The role of NRBCs count in critically ill neonates is confirmed by **Morton et al.,** who reported that among critically ill neonates, NRBCs are associated with significantly elevated mortality risk. although the median value for NRBCs count was found to be sometimes higher for non-survivors, this parameter did not have good discriminative ability for predicting mortality in all the study population, probably because of the great variability in NRBCs and the small number of cases [9].

For NICU patients, elevated NRBCs counts should prompt critical evaluation of patient status and care plans. As higher NRBC values are associated with shorter time to

mortality in addition to increased mortality, the trajectory of NRBCs counts may also be clinically

useful [9].

Furthermore, we found that NRBCs count with more than 10 per 100 WBCs, had high sensitivity in predicting complications of asphyxia[9].

Similar results were also selected for absolute NRBCs count.It has been documented that most of acute and chronic condition could increase NRBCs count through boosting the erythropoietic activity [1].Previous studies have been suggested NRBCs as hematopoietic marker in neonates and also its relationship with intrauterine hypoxia [10-12]. In hypoxic conditions such as in perinatal asphyxia, a compensatory response is created as increased erythropoiesis [13].

Another point of discussion is the positive association between the NRBCs count and the need for NICU admission, which was documented in previous studies [14].

Accordingly, NRBCs may be encompassed among the early diagnostic and prognostic markers for NICU patients. Further studies are needed to assess trends in NRBCs values for critically ill neonates. Previous studies identified physiological stressors, such as acidemia, hypoxic-ischemic encephalopathy, and respiratory distress etiologies, which were specifically associated with elevation in nucleated red blood cells count. This study extends the association between elevated nucleated red blood cells counts and illness severity in the preterm infants and term infants in the first days of life characterized in various studies [9].

The major limitations to our study were limited time of the study, limited number of cases and limited follow up duration.

Conclusions:

Nucleated red blood cells monitoring is a promising affordable marker indicating the exposure to stress or severe illness in perinatal period. It can be useful in follow up of the

critically ill infants during the first days of life together with the other routine investigations and clinical evaluation.

References

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Table 1: Demographic data of the studied cases.

Data	N (%) of 120 cases
Gestational age, week:	
<28	0
≥28, <32	2 (1.66%)
≥32, <36	51 (42.5%)
≥36	69 (57.5%)
Sex:	
Male	60 (50%)
Female	60 (50%)
Birth weight, gram:	
500-999	0
1000-1499	12 (10%)
1500-2499	28 (23.3%)
≥2500	80 (66.6%)
Duration of admission, days:	
1	5 (4.1%)
2-10	41 (34.16%)
>10	74 (61.6%)
NICU discharge status:	
Alive	60 (50%)
Deceased	60 (50%)

Table 2: Analysis of neonates with NRBCs in 1st 24 hours

Number of cases with detectable level of NRBCs in 1st 24 hours	49 (40.8%)
Number of cases with high NRBCs in 1st 24hours beyond normal range (>10/100 WBCs)	32 (65.3% of 49 cases with detectable levels of NRBCs) 26.6 % of all 120 cases of the study
Discharged Alive	4 (12.5% of 32 cases with NRBCs number beyond normal range)
Deceased	28 (87.5 % of 32 cases with NRBCs number beyond normal range)

NRBCs: Nucleated red blood cells, WBCs: White blood cells

Table 3:Relation between NRBCs appearance in 1st 24 hours & neonatal outcome

NRBS		Relation to neonatal outcome						
		Discharged good (N=60)		Deceased (N=60)		Total	chi square ($\chi 2$)	P value
		N	%	n	%			
NRBCS 1st day	Present	10	20.4	39	79.6	49	29.008	0.001*
	Absent	50	70.4	21	29.6	71		
NRBS Range	Up to 10 (normal)	56	63.6	32	36.4	88	24.545	0.001*
	>10 (abnormal)	4	12.5	28	87.5	32		

Table 4: Relation between NRBCs presence between 2^{nd} -10^{th} day & neonatal outcome

NRBCs		Relation to neonatal outcome						
		Discharged good(N=60)		Deceased (N=60)		Total	chi square ($\chi 2$)	P value
		n	%	n	%			
NRBCs 2^{nd} -10^{th} day	Present	18	34.6%	34	65.4%	52	8.688	0.003*
	Absent	42	61.8%	26	38.2%	68		
	Up to 10 (normal)	58	57.4	43	42.6	101	14.070	0.001*
	>10 (abnormal)	2	10.5	17	89.5	19		

Table 5: Relation between NRBCs presence after 10th day & neonatal outcome

NRBCs		Relation to neonatal outcome						
		Discharged (good)(N=60)		Deceased (N=60)		Total	chi square (χ2)	P value
		N	%	N	%			
NRBCs	Present	1	3.2	30	96.8	31	36.578	0.001*
	Absent	59	66.3	30	33.7	89		