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Original Article

Correlation between nucleated red blood cells and pulse oxygen saturation in neonatal asphyxia

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Abstract

Background Neonatal asphyxia is the major cause of neonatal death at a rate of approximately 23%. The incidence of asphyxia is higher in developing countries, due to limited availability of perinatology facilities. Hypoxia due to asphyxia is characterized by low pulse oxygen saturation (SpO_2), which basic health care facilities are unable to monitor. The number of nucleated red blood cells (nRBCs) in asphyxia increases in order to compensate for the hypoxia. Few studies have reported on nRBCs as they relate to pulse oxygen saturation in neonatal asphyxia.

Objective To assess for a correlation between nRBCs and pulse oxygen saturation in neonatal asphyxia.

Methods In this cross-sectional study, asphyxia was assessed by way of Apgar scores; pulse oxygen saturation was monitored by pulse oximetry; and nRBCs were determined by blood smears. Statistical analysis used was Spearman's test.

Results Subjects were 41 neonates with asphyxia, 15 of whom had 5th minute Apgar scores ≤ 6. Subjects with Apgar scores ≤ 6 had significantly higher umbilical venous nRBC counts [20.0 (SD 13.09) /100 white blood cell] than subjects with Apgar score > 6 [8.81 (SD 8.71) /100 white blood cell]; (P = 0.004). Subjects with Apgar ≤ 6 had significantly lower 5th minute SpO_2 values [76.46 (SD 6.17) %] than subjects with Apgar scores > 6 [87.03 (SD 6.29)]; (P<0.0001). Spearmans' test revealed a significant correlation between higher nRBC counts and lower pulse oxygen saturation (r = -0.804; P<0.0001).

Conclusion In asphyxia neonatorum there is a correlation between umbilical vein nRBC counts and the 5th minute SpO_2. As such, we recommend using nRBC examinations to predict pulse oxygen saturation as a means to assess the severity of hypoxia in peripheral areas where pulse oximetry machines may be unavailable. **[Paediatr Indones. 2014;54:314-7.]**

Keywords: *nRBC, pulse oxygen saturation, asphyxia*

Neonatal asphyxia is the major cause of neonatal deaths, more than infection and prematurity.[1-3] Approximately 23% of neonatal deaths are caused by asphyxia.[4] The incidence of asphyxia is 1-1.5% and correlates with gestational age and birth weight. The incidence is higher in developing countries due to the limited availability of antenatal and perinatal facilities.[1]

Asphyxia is defined as spontaneous and regular respiratory failure within a short time after birth. Hypoxia, hypercapnea, and acidosis are characteristics of asphyxia that may cause circulatory disturbances.[2] Hypoxia leads to multiple organ failure and death, if not well treated.[1,5,6] Oxygen saturation describes the percentage of hemoglobin binding sites in the bloodstream occupied by oxygen. Hypoxia in asphyxia can be manifested as low oxygen saturation.[2,6] Pulse oxygen saturation (SpO_2) is measured non-invasively using pulse oxymetry. Pulse oxymetry results can be influenced by intravascular volume, vasoconstriction, environmental lightning, and movement of the extremity to which the probe is attached.[7]

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Nucleated red blood cells (nRBC) are immature red blood cells that have nuclei and circulate in the bloodstream. The number of nRBCs varies in healthy term infants, but is generally not more than 10/100 white blood cells (WBC). In hypoxia, the number of nRBCs increases through an erythropoietin-mediated mechanism to compensate for oxygen demand.[9]

Newborn SpO_2 measurements using pulse oximetry cannot be done in health care centers with limited facilities. However, bloodsmear examinations for nRBC measurements are simple and can be done even in limited health facilities.[8] Few studies have been done on nRBC and oxygen saturation. The aim of this study was to assess for a correlation between nRBC and peripheral oxygen saturation in neonatal asphyxia.

Methods

This cross-sectional study was conducted in Dr Kariadi Hospital from September 2012 to January 2013. The study was approved by the Medical Research Ethics Committee of Diponegoro University/Dr. Kariadi Hospital in Semarang.

Consecutive sampling was conducted to obtain our 41 neonates. The inclusion criteria were moderate or severe asphyxia, gestational age ≥ 35 weeks, and without hypothermia, hypovolemic shock, or intrauterine growth restriction (IUGR). Subjects had Hb > 12.0 g/dL, WBC 5,000-25,000/mm^3, and mothers who did not smoke, or had diabetes, anemia, leukemia, chorioamnionitis, or antepartum bleeding. Exclusion criteria were ABO or rhesus incompatibility and cyanotic congenital heart disease.

Subjects underwent SpO_2 measurements at the 5th minute. Umbilical venous bloodsmears was performed to measure nRBCs soon after birth. A clinical pathogologist examined the nRBCs and was blinded to subjects' SpO2 values.

Independent sample T-test was used to assess for differences in nRBC counts between subjects with 5th minute Apgar scores ≤ 6 and > 6. Spearman's test was used to assess for a correlation between nRBC counts and 5th minute SpO_2. Statistical analysis was done with a computer program.

Table 1. Characteristics of subjects

Characteristics	n=41
Gender, n (%)	
Male	18 (43.9)
Female	23 (56.1)
Mode of delivery, n (%)	
Vaginal, unassisted	9 (22)
Assisted	32 (78)
Median gestational age (range), weeks	37 (35-41)
Mean birth weight (SD), grams	2,635.36 (554.25)
5th minute Apgar score, n (%)	
≤6	15 (36.6)
>6	26 (63.4)

Results

From 62 newborns with asphyxia, 41 neonates fulfilled the inclusion criteria. Subjects consisted of 18 (43.9%) males, and 9 (22.0%) underwent vaginal, unassisted deliveries. Subjects' mean birth weight was 2,635.36 (SD 554.25) grams. There were 15 (36.6%) subjects with Apgar scores ≤ 6 and 26 (63.4%) subjects

Table 2. Subjects' characteristics based on 5th minute Apgar scores

Characteristics	Apgar ≤ 6 (n=15)	Apgar > 6 (n=26)	P value
Gender, n			
Male	6	12	0.702¥
Female	9	14	
Mode of delivery, n			
Vaginal, unassisted	3	6	1.000 Φ
Assisted delivery	12	20	
Mean gestational age (SD), weeks	37.00 (1.69)	36.57 (1.39)	0.465Ω
Mean maternal Hb (SD), g/dL	11.65 (0.52)	11.89 (0.93)	0.297Ω
Mean maternal WBC (SD), /mm^3	13,745 (3,540)	12,200 (3,807)	0.158 α
Mean birth weight (SD), grams	2,678 (601)	2,611 (537)	0.723 α
Mean newborn Hb (SD), g/dL	13.81 (1.51)	13.49 (1.23)	0.695Ω
Mean newborn WBC (SD), /mm^3	15,220 (5342)	15,248 (5,707)	0.987 α
Mean nRBC (SD), /100 WBC	20.00 (13.09)	8.81 (8.71)	0.004 α
Mean SpO_2 (SD), %	76.46 (6.17)	87.03 (6.29)	<0.0001Ω

¥ Chi square test, Fischer's exact test, Ω Mann Whitney test, α Independent sample T-test

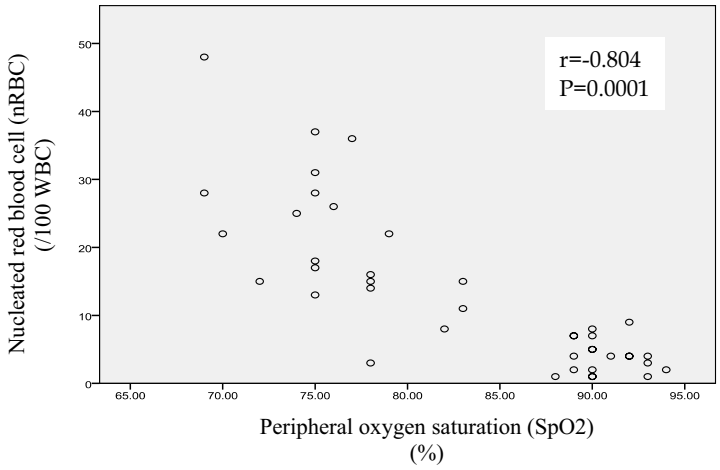

Figure 1. Scatter diagram of nRBC distribution

with Apgar scores > 6. Subjects' characteristics are described in **Table 1**.

Subjects were divided into two groups based on their 5th minute Apgar scores: Apgar scores ≤ 6 (n=15) and > 6 (n=26). There were no significant differences in gender, delivery method, gestational age, maternal Hb values, maternal WBC counts, newborn Hb values, and newborn WBC counts between the two groups. Subjects with Apgar scores ≤ 6 had significantly higher umbilical venous nRBC counts [20 (SD 13.09) /100 WBC] than subjects with Apgar scores > 6 [8.81 (SD 8.71) /100 WBC]; (P = 0.004). Subjects with Apgar scores ≤ 6 had significantly lower 5th minute SpO_2 values [76.46 (SD 6.17) %] than subjects with Apgar scores > 6 [87.03 (SD 6.29) %]; (P < 0.0001) **(Table 2)**.

Spearman's correlation test revealed a significant correlation between nRBC and SpO_2 (r = -0.804; P=0.0001). The nRBC count distribution with regards to SpO2 is shown in **Figure 1**.

Discussion

We included 41 neonates during the study period from September 2012 to January 2013. The subjects were similar to those in a study by Dawson *et al*. who found SpO_2 reference values in newborns with mean birth weight of 2,970 (SD 918) grams and mean gestational age of 38 (SD 4) weeks.[10] We did not exclude ≥ 35 week-preterm infants, due to the small number of newborns with asphyxia during the study period and the subjectivity of the first day of last menstrual period calculation, New Ballard score examination, and there were no significant differences in hematology results between late-preterm and fullterm newborns.[11] Low birth weight newborns were also not excluded due to the majority of preterm infants had birth weight < 2,500 grams, but we excluded those with IUGR.

We found no significant difference in maternal WBC counts between subjects with 5th minute Apgar scores of ≤ 6 and > 6. We excluded newborns from mothers with higher than normal WBC counts due to the effect of infection and leukocytosis in nRBC calculations.[8,12] Only newborns with normal Hb values were included in this study, in order to minimize the effect of anemia on nRBC and SpO_2 values.[8,13] There were no differences in Hb values between groups with 5th minute Apgar score ≤ 6 and > 6. Newborns with higher or lower than normal WBC counts were also excluded due to leukocytosis or leukopenia, and the effect of newborn infections on nRBC calculations.[8,14] There were no differences in WBC counts between the Apgar score ≤ 6 and > 6 groups.

Subjects with the 5th minute Apgar scores ≤ 6 had higher nRBC counts than subjects with the 5th minute Apgar scores > 6. A previous study found that increased nRBC count correlated with acidosis severity and low Apgar score.[15] Another study also found a negative, strong correlation between nRBC and 1st minute Apgar score and umbilical artery pH.[16, 17] Neonates with 5th minute Apgar scores

≤ 6 had significantly lower SpO_2 values than they with 5th minute Apgar scores > 6. A study compared SpO_2 in 90 neonates with asphyxia and 30 control found that the 3rd and the 5th minute SpO_2 levels in neonates with asphyxia were lower compared to the controls.[18] We found a strong correlation between increased nRBC count and low SpO_2, due to hypoxia. A study found that increased nRBC counts can be caused by acute or chronic hypoxia, which induces erythropoiesis and the release of erythrocytes and nRBC from the bone marrow to circulation.[8] A previous study found increased nRBC counts in newborns with Apgar scores ≤6,[19] another study found a correlation between nRBC count with low 1st minute Apgar scores and the severity of asphyxia.[17]

A limitation of our study was that we did not exclude late and preterm newborns, due to the small number of newborns with asphyxia during the study period. Also, the nRBC count was performed by only one expert. (KAPPA test was not done in this study.)

In conclusion, there is a significant inverse correlation between umbilical vein nRBC counts and the 5th minute SpO_2 in asphyxia neonatorum. Nucleated RBC counts in newborns with 5th minute Apgar scores of ≤ 6 are higher compared to newborns with the 5th minute Apgar scores of > 6. As such, umbilical vein nRBC counts can be performed in newborns with asphyxia to predict SpO_2 and the severity of hypoxia in rural areas with limited access to oxygen saturation monitor.

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