Red Cell Distribution Width as a Predictor of Persistent Pulmonary Hypertension of the Newborn

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 Persistent pulmonary hypertension of the newborn (PPHN) with the definition of persistent right-to-left shunting of blood through foramen ovale and ductus arteriosus or both after birth leads to severe hypoxemia. It occurs in 10% of term and near-term infants. This is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates. It is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates.

Keywords
► persistent pulmonary hypertension of the newborn
► hypoxemia
► neonatal intensive care unit
► red cell distribution width

Abstract

Objective Persistent pulmonary hypertension of the newborn (PPHN) is a critical condition with high mortality and morbidity rates in neonatal intensive care unit (NICU) admitted neonates due to severe hypoxemia. The aim of this study was to evaluate red cell distribution width (RDW) as a biomarker of hypoxemia and determine the optimal cutoff point of RDW for identifying neonates with PPHN.

Study Design All PPHN diagnosed, NICU admitted term infants with hypoxemia after birth from May 2014 to September 2016 were enrolled as case control and healthy term infants with nonhemolytic jaundice who were admitted for phototherapy on the second or third day of birth were the control group. Blood samples were collected. Multiple logistic regression modeling was used to examine the association between PPHN and RDW.

Result Receiver-operating characteristics (ROC) curve analysis was used to determine the optimal cutoff point of RDW for identifying neonates with PPHN. RDW was higher in the PPHN group compared with the control group (p < 0.001). Significant predictors of PPHN were mother’s underlying disease (p = 0.01) and RDW (p < 0.001). The optimal RDW cut point for prediction of PPHN by the ROC curve analysis was 17.9 (sensitivity = 85.71%). RDW’s area under the curve was 0.9197 (p < 0.001).

Conclusion RDW may be a simple, valuable, accessible marker for predicting PPHN before performing echocardiography in hypoxemic NICU admitted neonates.
values indicate an increase in variations of red blood cell (RBC) volume. Previously, this was calculated by dividing the standard deviation of RBC volume to the mean corpuscular volume and multiplying the product by 100, nowadays this is automatically analyzed.6

Recently, RDW as an accessible predictive marker of mortality in different diseases in adults including coronary artery,5 heart failure,6,7 stroke,8 bacterial infections and septic shock,9,10 and diabetes11 has been studied. Moreover, the correlation between RDW and mortality in older adult patients has been demonstrated.12,13 Though the mechanism of increased RDW is not known, inflammatory processes14 and hypoxic events15 are suggested as the mechanisms of these occurrences.

Limited information is available about the association of RDW and neonatal diseases. RDW has been demonstrated as a prognostic tool in neonatal sepsis.16

The aim of this study was to evaluate the relationship between RDW and PPHN as a biomarker of hypoxemia and to determine whether it can serve as an accessible marker for prediction of PPHN in NICU admitted neonates and determine the optimal cutoff point of RDW for identifying PPHN in neonates. According to our knowledge, this is the first time that this correlation has been studied during neonatal periods.

Methods

Study Design and Population

This case–control study was conducted during 28 months from May 2014 to September 2016 on all full-term newborns (with the gestational ages of ≥37 weeks) with diagnosis of PPHN who were admitted to the NICU of Arash Hospital in Tehran, soon after birth. These neonates constituted the case group. Healthy term infants with unconjugated hyperbilirubinemia, who were admitted to our neonatal ward only for phototherapy on the second or third day after birth, were included as control group. The study was approved by the Research Committee of Faculty of Medicine, Tehran University of Medical Sciences and the local ethical committee and written consent were obtained from parents before enrollment.

Inclusion criteria in the case group are all term infants with hypoxemia who were intubated within the first hour of birth with diagnosis of PPHN based on real-time echocardiography combined with Doppler flow imaging (echocardiography, MyLab7, Esaote, Italy). This was done by neonatal cardiologists within 24 hours after birth. Estimation of right ventricle pressure (RVP), using assessments of tricuspid regurgitation jet is compared with systemic blood pressure and estimated RVP greater than one-half systemic blood pressure suggests PPHN. Inclusion criteria in control group are all healthy, full-term exclusively breast fed newborns with nonhemolytic jaundice.

Exclusion criteria are (1) infants born to mothers with chorioamnionitis, (2) major congenital abnormalities at birth, (3) preterm infants, (4) congenital heart disease, (5) early onset sepsis with positive blood culture, (6) congenital diaphragmatic hernia, (7) hemolytic anemia, (8) hypoglycemia, and (9) polycythemia.

Mother’s underlying disease during pregnancy (diabetes mellitus, urinary tract infection, preeclampsia, and hydrops) and drug consumption during pregnancy were also registered. To avoid any confounding effects, blood samples for the CBC count (including RDW) were collected at the time of NICU admission. CBC count was calculated by the automated hematology analyzer XE-1200 (Sysmex, Japan). Arterial blood gas analyses and other tests such as serum biochemical analysis were also conducted. Blood gas analyses were performed in Radiometer device ABL 900 flex sensor cassette and solution package. In this study, metabolic acidosis was defined as pH < 7.2 and base deficit in the extracellular fluid > 12.0 mmol/L in umbilical artery according to previous studies.17

Laboratory tests for management of hyperbilirubinemia in term infants including CBC count were also conducted based on the American Academy of Pediatrics guideline.18

RDW was reported as the coefficient of variation (percentage) of RBC volume as measured with an automated analyzer (Beckman Coulter LH780 hematology analyzer; Beckman Coulter, Brea, CA).

Statistical Analysis

Categorical and continuous variables were expressed as number (percentage) and mean ± (standard deviation [SD]), respectively. A chi-square test or Fisher’s exact test was performed to compare categorical variables. Student’s t-test was used to compare parametric continuous variables. The Mann–Whitney’s U-test was used to compare nonparametric continuous variables. Multiple logistic regression modeling was used to examine the association between PPHN and RDW. Results are presented as odds ratio (OR) with 95% confidence intervals. Variables were considered in the model for adjustment in the following order: gestational age, gender, weight, the first- and fifth-minute Apgar score. Variable selection was done based on the available evidence about the PPHN risk factors as much as possible, without overlapping with other measurements to avoid collinearity in the logistic model. Since the data had several unbalanced and highly predictive risk factors (complete separation problem), the multiple logistic regression model was performed using firthlogit to examine possible association between outcome of interest (PPHN) and independent variables. The presence of the aforementioned problems in logistic regression models can result in bias of OR estimates away from 1. The firthlogit command did not use maximum log likelihood but penalized log likelihood instead of reducing bias.

Furthermore, receiver-operating characteristics (ROC) curve analysis was used to determine the optimal cutoff point of RDW for identifying neonates with PPHN. Areas under the curve (AUC) and its standard error were calculated. Data analysis was undertaken using Stata statistical software, release 13.0 (StataCorp, College Station, TX).

Results

A total of 652 preterm and term infants were admitted to our hospital, of which 138 (25%) fulfilled the eligible criteria, out of which 63 (11.4%) with diagnosis of PPHN were considered
as the study group, while 75 healthy term infants with unconjugated hyperbilirubinemia without hemolysis were included in the control group.

Clinical and demographic findings of both groups are described in Table 1. There was no significant difference between maternal age in the case group (25.78 ± 5.17) and control group (25.64 ± 6.25). In the case group, 25 (39.7%) of mothers had underlying diseases, 10 of which were taking levothyroxine due to hypothyroidism, 3 were taking insulin, and the others were on a diabetic diet.

In the case group, the mean (SD) Apgar score was 5.73 ± 2.49 in the first minute and 7.69 ± 2.13 in the fifth minute. The mean (SD) weight of cases was 2,896.11 ± 525.42 which was significantly lower than the mean (SD) weight of controls, 3,190.6 ± 564.92 (p = 0.002). Compared with neonates with PPHN, neonates without PPHN had higher levels of first-minute Apgar score (p = 0.02) and fifth-minute Apgar score (<0.001). The etiologies of the PPHN were pneumonia (n = 18), birth asphyxia (n = 17), respiratory distress syndrome (n = 11), meconium aspiration syndrome (n = 9), and malignant transient tachypnea of the newborn (n = 8). Three deaths were observed in the study group while there was no death in the control group. RDW was higher in cases in comparison with the control group (19.99 ± 2 vs. 16.32 ± 2.19, respectively; p < 0.001).

The optimal RDW cut point for prediction of PPHN by the ROC curve analysis was 17.9, which yielded sensitivity 85.71% and specificity 85.33%. Overall, the AUC of RDW was 0.9197 (95% CI: 0.8743–0.965, p < 0.001) (Fig. 1). Positive likelihood ratio and negative likelihood ratio were 5.84 and 0.16, respectively.

### Discussion

PPHN is a critical condition with high mortality and morbidity rates in NICU admitted neonates due to severe hypoxemia. Physical examination is usually associated with some restrictions for diagnosis of PPHN. Access to RDW index before carrying out echocardiography in these situations will be accompanied by early intervention and aggressive treatment.

Based on our knowledge, this is the first time that the value of RDW as a predictor marker of critical diseases has been studied during the neonatal period excluding sepsis.

The present study evaluated the correlation of RDW and PPHN and shows that RDW, as a simple accessible test, increases during PPHN with any etiologies in comparison with healthy term infants. The RDW reference interval in

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**Table 1** Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n = 63)</th>
<th>Control (n = 75)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>37.58 ± 1.12</td>
<td>37.69 ± 0.79</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2,896.11 ± 525.42</td>
<td>3,190.6 ± 564.92</td>
<td>0.002</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>19.99 ± 2</td>
<td>16.32 ± 2.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boy</td>
<td>28 (44.4)</td>
<td>39 (52)</td>
<td>0.37</td>
</tr>
<tr>
<td>Girl</td>
<td>35 (55.6)</td>
<td>36 (48)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Adjusted OR for relationship of PPHN and its predictors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted OR</th>
<th>SE</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>1.02</td>
<td>0.27</td>
<td>0.26–2.03</td>
<td>0.56</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.99</td>
<td>0.0004</td>
<td>0.99–1.0005</td>
<td>0.91</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>2.11</td>
<td>0.31</td>
<td>1.57–2.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.74</td>
<td>0.38</td>
<td>0.26–2.03</td>
<td>0.56</td>
</tr>
<tr>
<td>Mother’s underlying disease</td>
<td>41.71</td>
<td>61.98</td>
<td>2.26–767.52</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; PPHN, persistent pulmonary hypertension of the newborn; RDW, red blood cell distribution width; SE, standard error.

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**Fig. 1** ROC curve analysis for persistent pulmonary hypertension. ROC, receiver-operating characteristic.
term healthy infants at birth was reported between 15.5 and 20% by Christensen et al with the lower reference limit of 15.5%19. In a retrospective study which was done by Garofoli et al, mean (± SD) RDW values measured within the first 3 days after birth were 15.65 ± 1.18% in full-term newborns.20 In our study, the mean of RDW in term healthy infants was 16.32 ± 2.19 which was the same as Chen et al’s study (16.04 ± 1.25).16

This study also shows that the elevation of the RDW index can be an independent predictor of PPHN. Recently, a prognostic role of RDW in pulmonary hypertension21 and follow-up in ICU newborns and the severity of obstructive sleep apnea and carotid intima22 have been suggested. Although the mechanism of increased RDW is not known, inflammatory processes16 and hypoxic events15 are suggested as the mechanisms of these occurrences.

Based on the YCas et al’s erythropoietin risk-pathway model, hypoxia, due to any disease, increases the RDW index and this can be a biomarker of hypoxemia in adults.15 PPHN due to persistent right-to-left shunting of blood through foramen ovale and ductus arteriosus or both after birth leads to severe systemic hypoxemia. As a result, the most probable hypothesis is that, in PPHN infants, persistent hypoxia induces an increase in RDW. The predictive role of RDW as an accessible predictor of mortality in different diseases in adults has been studied as well.

In the study by Aksoy et al, a negative correlation was observed between RDW and gestational age (r = −0.51; p < 0.001). In their study, higher RDW in premature infants within the first 3 days of their life has also been associated with increased rates of mortality (p < 0.0001) and late-onset sepsis (p < 0.005).24 However, in the study by Aksoy et al, RDW did not predict mortality in very low birth weight infants. Chen et al showed that there was a negative correlation between the neonatal critical illness score and RDW, while there was a positive correlation with mortality rate in the septic shock group (p < 0.001). The increase of the RDW index was associated with the severity of diseases as a result, they considered RDW as a probable prognostic factor of neonatal sepsis.16 In their study, they did not mention the sensitivity and the specificity of RDW and the optimal RDW cut point.

In our study, there was no relationship between RDW and the mortality rate; this might be due to the low number of dead infants.

The optimal RDW cut point for prediction of PPHN by the ROC curve analysis was 17.9, which yielded sensitivity 85.71% with the AUC of RDW 0.9197 (p < 0.001). There is no previous study during neonatal periods that can be compared with our study. In the study by Abul et al, the AUC of RDW for prediction of chronic thromboembolic pulmonary hypertension after pulmonary embolism was 0.735 with 75% sensitivity and they considered RDW as an independent predictor of chronic thromboembolic pulmonary hypertension in adults.25 In comparison with Abul et al’s study, it seems that RDW might help the prediction of PPHN with a higher sensitivity value.

**Study Limitations and Strengths**

A case–control and cross-sectional study was the main limitation of this study. It is better if cohort studies in multicenter hospitals are done due to the very low incidence rate of this condition. Further investigations need to evaluate this marker for each disease separately and to estimate the morbidity outcome during the neonatal period.

Despite the limitations, this was the first study that was conducted during neonatal periods in hypoxemic events, thus distinguishing this article from others.

**Conclusion**

Based on this study, RDW as a simple, accessible marker, with a high predictable value may be used to predict PPHN before performing echocardiography in NICU admitted neonates with hypoxemia for better and faster treatment.

**Funding**

None.

**Conflict of Interest**

None.

**References**