INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 64
2022 November; 29(1): 64-68
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Comparison of Reticulocyte Hemoglobin Equivalent Levels between Low and Normal Birth Weight Newborns

Resvi Livia, Fajar Wasilah, Leni Lismayanti

Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin Hospital, Bandung, Indonesia. E-mail: resvilivia.dr@gmail.com

ABSTRACT

Low Birth Weight (LBW) newborns face a risk of iron deficiency. Iron deficiency hinders growth, and motoric, and cognitive development. Newborns with LBW sometimes suffer from inflammation, which affects the commonly used iron measurements. Reticulocyte hemoglobin equivalent (Ret-He) is considered a potential tool to measure iron profile because it measures functional iron, and it is not affected by inflammation. This study compared the Ret-He in LBW and normal birth weight newborns. This cross-sectional study was done retrospectively by observing and comparing the hematology data of newborns from November to December 2019. The difference in Ret-He level was assessed using a non-parametric test. Out of 70 newborns, 26 were normal and 44 were LBW. The proportion of LBW newborns with anemia was higher than the proportion of normal ones (29.6% vs. 7.7%, p=0.03). The median value of Ret-He in LBW was lower compared to normal birth weight (32.6 vs. 33.3 pg, p=0.09), however, the values were still within the normal limits. Five from 70 of these newborns' Ret-He levels were under the reference range (7.14%). There was found that CRP levels were higher in LBW newborns than normal ones (5.6% vs. 5%, p=0.98). There was a positive correlation between Ret-He and the birth weight of the newborns (r= 0.34, p = <0.01). There was no significant difference in Ret-He levels of LBW compared to normal babies. Further research is needed with a larger sample size to better assess the association of Ret-He and iron profiles in newborns.

Keywords: Newborn, low birth weight, preterm, Ret-He

INTRODUCTION

Low Birth Weight (LBW) is defined as babies whose birth weight is lower than 2500 grams. These babies have a higher morbidity and mortality rate and have a greater risk of developing health problems such as iron deficiency and anemia.[1,2]

Globally, it is estimated that 7-15% of newborns have low birth weight and this prevalence is even higher in developing countries.[3,4] Based on data obtained from the Indonesian Health Ministry in 2018 (Riset Kesehatan Dasar), the proportion of low birth weight in Indonesia is 6.2%.[5]

Stored iron levels in the fetus are proportional to its birth weight. Low birth weight babies have a greater risk for iron deficiency or iron deficiency anemia, therefore LBW and premature babies require higher iron than the aterm ones.[6] Iron transfer from the mother to fetus during pregnancy is terminated when the preterm baby is born.[3,7] Iron Deficiency Anemia (IDA) is the most common cause of anemia. This anemia can hinder growth, motor, and cognitive development, raise infection susceptibility, and increase the risk of Attention Deficit Hyperactivity Disorder (ADHD).[8-10] Early detection of IDA in the golden period of child growth and development is very important to evaluate early iron therapy indication and prevention efforts. However, finding a good parameter to assess iron status in newborns is still challenging, especially in babies who require intensive treatment (NICU patients).[11] Currently, there is no gold standard for the examination of iron deficiency in newborns.[12]

Reticulocyte hemoglobin equivalent (Ret-He) or Reticulocyte hemoglobin content (CHr) has emerged as a potential iron status examination. The Ret-He measures the amount of hemoglobin contained in reticulocytes. Therefore Ret-He examination indirectly measures the amount of functional iron in red blood cell formation.[13,14] Decreased levels of Ret-He in iron deficiency appear earlier than the decrease of Hb, MCV, and MCH.[15] In addition, Ret-He examination can be simultaneously obtained with a complete hematological examination by using certain automatic hematology devices, so it does not require any extra samples or tubes unlike iron status examination using other parameters such as serum iron, Total Iron Binding Capacity (TIBC), and serum transferrin.[16] Unlike ferritin, Ret-He measurement is not affected by inflammatory conditions. There are

no significant confounding factors except for alpha or beta thalassemia and macrocytosis, making Ret-He or CHr a potentially better indicator of iron deficiency compared to biochemical parameters that are influenced by inflammation.[17] Previous studies in Indonesia regarding Ret-He and anemia in chronic kidney diseases reveal that Ret-He has a moderate correlation with serum iron and transferrin saturation.[18] The CHr parameter released by ADVIA 120 has been approved for clinical application by the Food and Drug Administration (FDA) in August 1997 in America.[19]

Assessment of Ret He levels in newborns could be a promising marker for the diagnosis and monitoring of iron deficiency in newborns, which have a golden period of growth and development. In addition, iron supplements among newborns have been recommended by the Indonesian Pediatrician Association (Ikatan Dokter Anak Indonesia/IDAI) and the World Health Organization (WHO).[20] Therefore, the main objective of this study was to determine the difference in Ret-He levels between low and normal birth weight newborns at Dr. Hasan Sadikin Hospital, Bandung, West Java.

METHODS

This study was an unpaired observational comparative study. Data were obtained retrospectively and cross-sectionally during November-December 2019 from the Laboratory Information System (LIS) and medical records. Ret-He levels were obtained from a complete hematology examination within 48 hours after birth using the Sysmex XN-1000 automatic hematology analyzer. The Ret-He measurement results were achieved by converting the mean of reticulocytes' scattered light signals into numeric values using a certain calculation equation developed by Sysmex. This new parameter was expressed in units of picograms (pg). C-reactive protein was examined simultaneously using Siemens EXL 200 Dimension. Maternal Hb results were taken before delivery to assess anemia. Statistical analysis was performed using SPSS ver 26.0. The Kolmogorov-Smirnov test was performed to determine the distribution of the data. The difference in the Ret-He median was analyzed using the Mann-Whitney test. The correlation between Ret-He and continuous variables was assessed using Spearman and Pearson correlation. P-value < 0.05 was considered statistically significant, with a 95% confidence interval.

RESULTS AND DISCUSSIONS

There were 94 complete blood counts and data of newborns within 48 hours of birth at Dr. Hasan Sadikin Hospital. Data from 70 babies were obtained for this study (Table 1). These newborns consisted of 26 (37.1%) babies with normal birth weight (≥ 2500 grams) and 44 (62.9%) with LBW (<2500 grams).

There were significant differences in gestational age, anemia conditions, and leucocyte counts between LBW and normal birth weight groups, while Ret-He levels, maternal anemia, platelet counts, and CRP levels had no significant differences. As many as 88.6% of LBW babies were born preterm (< 37 weeks), significantly different from normal birth weight babies, the majority of whom were born aterm (≥ 37 weeks). Anemia in LBW was more common than an anemia in normal birth weight (p=0.03). Almost all patients with normal birth weight babies had normal leucocyte counts, while in LBW babies one third were leucopenia (p=<0.01).

The median value of Ret-He levels in newborns with various characteristics (gender, birth weight, maturity, anemia status, and maternal anemia) can be seen in Table 2. It can be seen that there were significant differences in the levels of Ret-He between gestational age groups. While between gender, birth weights, and anemia conditions in mother and newborn, there were no significant differences between the two groups. Newborns with normal birth weight had higher Ret-He levels than LBW, but this difference was not significant (p=0.09). Babies from anemic mothers had lower Ret-He levels but were not significantly different from babies with non-anemic mothers. Newborn babies with anemia had lower Ret-He too, but they were not significantly different from non-anemic newborns. The percentage of newborns who had Ret-He results below the reference value (27.4-36 pg) in this study was 5/70 (7.14%).

The proportion of LBW babies in this study was higher than that of LBW babies in Indonesia, (62.9% vs 6.2%). The high proportion of LBW in Dr. Hasan Sadikin Hospital was probably due to the hospital being a referral hospital. A complete hematological examination containing Ret-He was only available for premature babies and/or babies with possible infection or sepsis.

In this study, premature babies contributed to 90% of LBW cases. This result is consistent with the study of Anil *et al.* that prematurity is one of the risk factors for LBW.[21] The decline of hemoglobin levels in premature babies occurred faster than in aterm

Table 1. Patient characteristics

	Normal Birth Weight (N=26)	Low Birth Weight (N=44)	p-value
Gender (%)[a]			
Male	13 (50)	16 (36.36)	0.26
Female	13 (50)	28 (63.64)	
Gestational age (%)[a]			
Term	23 (88.5)	5 (11.4)	**<0.01**
Preterm	3 (11.5)	39 (88.6)	
Anemia (g/dL)[a]			
No	24 (92.3)	31 (70.5)	**0.03**
Yes (<15)	2 (7.7)	13 (29.6)	
Leucocyte (10^3/µL)[b]			
Normal (9.1-38)	25 (96.2)	29 (65.9)	**<0.01**
Leukocytosis (=38)	1 (3.8)	1 (2.9)	
Leucopenia (<9.1)	0	14 (31.8)	
Thrombocyte (10^3/µL)[b]			
normal (84-478)	26 (100)	41 (93.2)	0.17
thrombocytosis (=478)	0	0	
thrombocytopenia (<84)	0	3 (6.8)	
Maternal anemia			
No	11 (42.3)	27 (65.9)	0.06
Yes (<11 g/dL)	15 (57.7)	14 (34.1)	
CRP (mg/dL)[φ]			
Normal (0.09-1.58)	15 (75)	26 (72.2)	0.98
High (>1.58)	1 (5)	2 (5.6)	
Low (<0.09)	4 (20)	8 (22.2)	
Ret-He (pg)[c]			
Median	33.3	32.6	0.09
Min-max	28.2-36.4	22.9-35.2	

Notes: φ: CRP (n=56) a: Chi-Square test b: ANOVA test c: Mann-Whitney test

Table 2. Ret-He level among newborns

	Median Ret-He (pg)	p-values
Gender		
Female	32.8	0.56
Male	32.7	
Birth weight		
=2500 g	33.3	0.09
< 2500 g	32.6	
Gestational age		
Aterm	33.4	**0.02**
Preterm	32.4	
Anemia		
No	32.7	0.38
Yes	32.3	
Maternal anemia		
No	32.9	0.47
Yes	32.6	

babies. This could explain why there were more anemic babies in LBW than in normal birth weight babies, even though the Ret-He levels differences were not significant. This study did not find a correlation between Hb and Ret-He levels (r=0.14, p=0.26; Figure 1). From this finding, can conclude that anemia in premature babies was probably not caused by iron deficiency. It seems that anemia due to prematurity was not correlated with Ret-He level and iron deficiency but more physiologic factors in nature or from external factors. Some of the physiologic contributors to anemia in premature babies are rapid body growth, shorten RBC lifespan, and low plasma erythropoietin levels among others. The non-physiologic contributors are laboratory blood loss, inadequate nutrient intake, and sepsis among others.[22]

Median Ret-He in normal birth weight was higher than LBW (33.3 vs. 32.6 pg, p=0.09). The normal

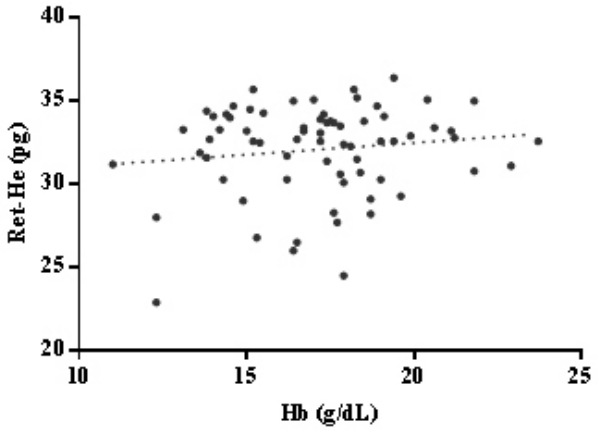

Figure 1. Correlation of Hb and Ret-He

Ret-He level from Lofving et al. was used as a reference range (27.4-36.0 pg).[23] In this study, found that there were 5 babies with low Ret-He, all of whom were LBW. There was find a moderate correlation between Ret-He and birth weight (Figure 2). As mentioned before the duration of pregnancy contributed to the amount of iron stored in newborns. Thus, the shorter gestational age is also related to the premature infant's low birth weight compromising the iron levels of the baby and causing anemia.[3]

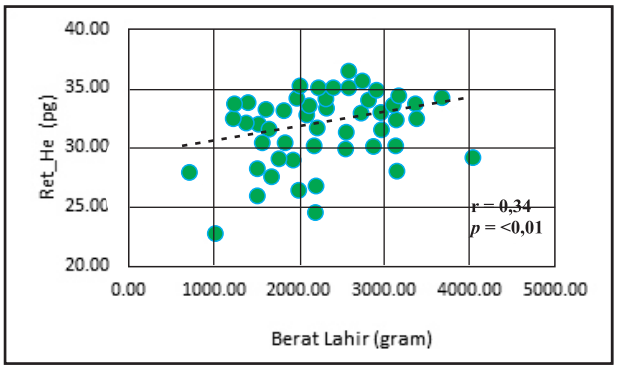

Figure 2. Correlation of birth weight and Ret-He

Forty percent among the low Ret-He level accompanied by an increasing CRP result (> 1.58 mg/dL). Higher levels of CRP as a marker of inflammation didn't cause a false increase in Ret-He results. The results of Ret-He in this study were supported by a study by Al-Ghananim *et al.* very low birth weight babies had a median Ret-He of 31.8 pg.[15] Lorenz *et al.* found that the mean Ret-He level in preterm babies who were born at 30-36 weeks gestation was 31.2 pg, consistent with this study.[24]

The percentage of anemic babies in the newborn from this study was 21.4%, which consisted of 86.7% babies with LBW and 13.3% with normal birth weight. Maternal anemia in this study was 41.4% consisting of 48.3% babies from the LBW group and 51.7% from normal birth weight. In this study, the Ret-He levels of the newborn from anemic mothers were lower than babies from mothers without anemia (32.6 vs. 32.9 pg), but the two groups were not significantly different (p=0.47). This study analyzed the correlation between maternal Hb and Ret-He levels of the newborn babies, and there was found no correlation between maternal Hb and Ret-He levels (r=0.002, p=0.86) (Figure 3). Anemia during pregnancy will increase the risk of iron deficiency in babies to the previous study.[25] However, in this study, anemia in the newborn was probably not caused by maternal anemia.

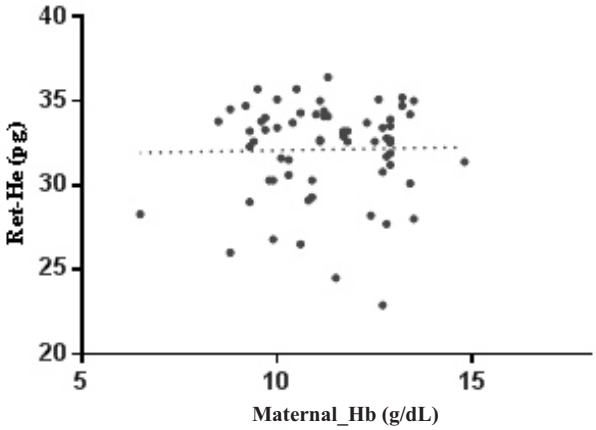

Figure 3. Correlation of maternal Hb and Ret-He

CONCLUSIONS AND SUGGESTIONS

There was no significant difference in the levels of Ret-He in LBW and normal birth weight babies in this study. This finding suggests that anemia in LBW babies was not caused by iron deficiency. Anemia due to prematurity was not correlated with Ret-He levels but more physiologic factors in nature like rapid body growth, shorten RBC lifespan, and low plasma erythropoietin levels. Further research is needed with a larger sample size in order to better assess the association of Ret-He and iron profiles in newborn.

REFERENCES

1. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci, 2019; 1450(1): 15-31.
2. Rocha G, Pereira S, Antunes-Sarmento J, Flôr-de-Lima

F, Soares H, Guimarães H. Early anemia and neonatal morbidity in extremely low birth-weight preterm infants. The Journal of Maternal-Fetal & Neonatal Medicine, 2021; 34(22): 3697-703.
3. Figueiredo ACMG, Gomes-Filho IS, Silva RB, Pereira PPS, Mata FAFD, et al. Maternal anemia and low birth weight: A systematic review and meta-analysis. Nutrients, 2018; 10(5): 601.
4. Khan JR, Islam MM, Awan N, Muurlink O. Analysis of low birth weight and its co-variants in Bangladesh based on a sub-sample from nationally representative survey. BMC Pediatr, 2018; 18(1): 100.
5. Kementerian Kesehatan Republik Indonesia. Riset Kesehatan Dasar (RISKESDAS). 2018. Available from: https://www.litbang.kemkes.go.id/laporan-riset-kesehatan-dasar-riskesdas/ (accessed Jan 9, 2020).
6. Moreno-Fernandez J, Ochoa JJ, Latunde-Dada GO, Diaz-Castro J. Iron deficiency and iron homeostasis in low birth weight preterm infants: A systematic review. Nutrients, 2019; 11(5): 1090.
7. Harmening DM. Clinical hematology and fundamentals of hemostasis. Philadelphia, PA: Davis, 2009; 117-28.
8. Warner MJ, Kamran MT. Iron deficiency anemia. StatPearls. Treasure Island (FL), StatPearls Publishing, 2021; 1-8.
9. Syed S, Kugathasan S, Kumar A, Prince J, Schoen BT, *et al.* Use of reticulocyte hemoglobin content in the assessment of iron deficiency in children with inflammatory bowel disease. Journal of Pediatric Gastroenterology and Nutrition, 2017; 64(5): 713-20.
10. Finkelstein JL, Herman HS, Guetterman HM, Peña?Rosas JP, Mehta S. Daily iron supplementation for prevention or treatment of iron deficiency anaemia in infants, children and adolescents. Cochrane Database Syst Rev, 2018; 2018(12): CD013227.
11. Sundararajan S, Rabe H. Prevention of iron deficiency anemia in infants and toddlers. Pediatric Research, 2021; 89(1): 63-73.
12. German K, Vu PT, Irvine JD, Juul SE. Trends in reticulocyte hemoglobin equivalent values in critically ill neonates, stratified by gestational age. J Perinatol, 2019; 39(9): 1268-74.
13. Ogawa C, Tsuchiya K, Maeda K. Reticulocyte hemoglobin content. Clinica Chimica Acta, 2020; 504: 138-45.
14. Buttarello M. Laboratory diagnosis of anemia: Are the old and new red cell parameters useful in classification and treatment, how?. Int Jnl Lab Hem, 2016; 38(S1): 123-32.
15. Al-Ghananim RT, Nalbant D, Schmidt RL, Cress GA, Zimmerman MB, Widness JA. Reticulocyte hemoglobin content during the first month of life in critically ill very low birth weight neonates differs from term infants, children, and adults. Journal of Clinical Laboratory Analysis, 2016; 30(4): 326-34.
16. Gelaw Y, Woldu B, Melku M. The role of reticulocyte hemoglobin content for diagnosis of iron deficiency and iron deficiency anemia, and monitoring of iron therapy: A literature review. Clinical Laboratory, 2019; 65: 12.
17. Jamnok J, Sanchaisuriya K, Chaitriphop C, Sanchaisuriya P, Fucharoen G, Fucharoen S. A new indicator derived from reticulocyte hemoglobin content for screening iron deficiency in an area prevalent for thalassemia. Lab Med, 2020; 51(5): 498-506.
18. Rovani F NA, Arif M. Analysis of ret-he in chronic kidney disease patients at Dr. Wahidin Sudirohusodo Hospital, Makassar. Indonesian Journal of Clinical Pathology and Medical Laboratory, 2018; 25(1): 7-10.
19. Khodaiji S. Hematopathology: Advances in understanding. New Delhi, Springer, 2019; 3-26.
20. Gatot DIP, Abdul Salam M. Suplemen besi untuk anak. Jakarta, Ikatan Dokter Anak Indonesia, 2011; 1-19.
21. Anil KC, Basel PL, Singh S. Low birth weight and its associated risk factors: Health facility-based case-control study. PLOS ONE. 2020;15(6):e0234907.
22. Ree IMC, Lopriore E. Updates in Neonatal Hematology: Causes, Risk Factors, and Management of Anemia and Thrombocytopenia. Hematology/Oncology Clinics of North America. 2019;33(3):521-32.
23. Löfving A, Domellöf M, Hellström-Westas L, Andersson O. Reference intervals for reticulocyte hemoglobin content in healthy infants. Pediatr Res. 2018;84(5):657-61.
24. Lorenz L, Peter A, Arand J, Springer F, Poets CF, Franz AR. Reference Ranges of Reticulocyte Haemoglobin Content in Preterm and Term Infants: A Retrospective Analysis. Neonatology. 2017;111(3):189-94.
25. Youssry MA, Radwan AM, Gebreel MA, Patel TAJOJoO, Gynecology. Prevalence of maternal anemia in pregnancy: the effect of maternal hemoglobin level on pregnancy and neonatal outcome. 2018;8(7):676-87.