

CORRELATION OF HEMOGLOBIN LEVELS WITH RETICULOCYTE HEMOGLOBIN EQUIVALENT (RET-HE) IN IRON DEFICIENCY ANEMIA PATIENTS

Dewi Astuti¹, Eva Ayu Maharani^{1*}, Iis Mulianasari², Ninik Sukartini²

¹ Health Polytechnic of Jakarta III

²Cipto Mangunkusumo Hospital

*evaayumarani@gmail.com

ABSTRACT

Iron deficiency anemia is caused by a lack of iron in the blood, which results in reduced hemoglobin (Hb) in the blood. Specific parameters such as ferritin, serum iron, and transferrin to help diagnose iron deficiency anemia are not always available in clinical laboratories and are quite expensive. The Hb level is a common parameter for anemia screening and reticulocyte hemoglobin equivalent (Ret-He) is one of the parameters in a hematology analyzer that can describe the availability of iron in the body but is not widely used. This study aims to determine the correlation between the Hb level and Ret-HE values in iron deficiency anemia. This research is an analytic observational study with a cross-sectional design, using medical record data on patients diagnosed with iron deficiency anemia. Sampling was carried out by purposive sampling method. The samples were calculated from the correlation calculation formula and obtained 76 data for the minimum sample. Based on inclusion criteria, 90 samples of data were obtained. The statistical test used bivariate analysis with the Pearson/Spearman correlation test. The correlation test was carried out at an α value of 0.05. The results showed that the average Ret-He value was 21.12 pg and the average Hb level was 9.8 g/dL. The data distribution showed an abnormal distribution, then The Spearman correlation test was carried out and obtained a P value = 0.000. The conclusion of the study, there is a positive correlation between Ret-He values and Hb levels in iron deficiency anemia patients. This shows that the Hb Level is directly proportional to the Ret-He value, the lower the Hb level in patients with iron deficiency anemia, then the lower the Ret-He value. This suggests that Ret-HE can also be used to determine iron deficiency anemia.

Keywords: hemoglobin; iron deficiency anemia; Ret-He

INTRODUCTION

Iron deficiency anemia is the most common type of anemia, especially in developing countries. More than one third of the world's population suffers from iron deficiency anemia which has very detrimental health impacts and quite serious social impacts (Pradiyadnya M and Suryani, 2017). Iron deficiency anemia is a type of anemia caused by a lack of iron in the blood, which inhibits the formation of erythrocytes which results in reduced hemoglobin (Harahap, 2018). Based on the basic health research Ministry of Health (2018), the prevalence of anemia among Indonesian people who lived in urban areas was 22.7% (Health research and development agency ministry of health of Indonesia, 2018).

The gold standard for the detection of Iron deficiency anemia is through the examination of bone marrow, however, this examination is invasive and quite expensive. Ferritin, serum iron, transferrin saturation, and TIBC can be an alternative examination for iron deficiency anemia, but these tests may be influenced by several factors and conditions. Low serum iron levels are not only found in iron deficiency anemia but can also be found in anemia caused by chronic disease. Examination of ferritin and transferrin saturation can be influenced by biological variations, such as diurnal variations and ferritin as an acute phase protein which can be affected by the presence of an inflammatory reaction (Primiastanti, 2013;Bain, Barbara; Bates, Imelda; Laffan, 2017;Uçar *et al.*, 2019).

The hemoglobin examination is a common parameter for anemia screening. Based on WHO standards, the concentration of Hb less than 12 g/dL is a sign of anemia (Aggarwal *et al.*, 2020). Low Hb levels cannot specifically identify the type of iron deficiency anemia, although in general, Hb levels in patients with iron deficiency anemia are below normal values (WHO, 2011). Therefore, other supporting parameters that are easy to apply are needed. The reticulocyte hemoglobin equivalent parameter (Ret-HE) is one of the parameters in the hematology analyzer which reflect the hemoglobin content of reticulocytes that have just been released from the bone marrow. Reticulocytes are the erythrocyte precursor cells. Measurement of hemoglobin in the reticulocyte can describe the availability of iron for the formation of red blood cells and the quality of red blood cells. Therefore, Ret-HE can be used to detect the iron deficiency of anemia earlier compared to Hb concentration (Pramantik, Ratnaningsih and Mulyono, 2016;Sysmex, 2023). Previous studies concluded that Ret-HE can be used as a parameter for assessing iron status in hemodialysis patients with chronic kidney disease. (Andriani, 2013). Another study in reproductive women concluded that Ret-HE can differentiate between the iron deficiency anemia group and without the iron deficiency anemia (Primiastanti, 2013). In this study, we analyzed

the correlation between Ret-HE value and hemoglobin concentration in patients with iron deficiency anemia as an alternative parameter to detect iron deficiency anemia with the advantage of more efficient in its procedure compared to other tests such as transferrin, TIBC and ferritin.

METHOD

This research is an analytic observational study with a cross-sectional design. The study was conducted using medical records data on patients diagnosed with iron deficiency anemia at Cipto Mangunkusumo Hospital. The data was taken comes from February to June 2022 period. Sampling was carried out by purposive sampling method. The samples were calculated based on correlation calculation formula as following.

$$n = \left\{ \frac{(z\alpha + z\beta)}{0,5 \ln \left[\frac{(1+r)}{(1-r)} \right]} \right\}^2 + 3 = \left\{ \frac{(1,96 + 1,65)}{0,5 \ln \left[\frac{(1+0,400)}{(1-0,400)} \right]} \right\}^2 + 3 = 76$$

Based on the calculation, a minimum sample size of 76 was obtained. Sampling was determined based on inclusion criteria, namely data on patients with stage 1 iron deficiency anemia with ferritin levels less than 20 ng/mL. The variables to be measured are data on Hb levels and Ret-He values in patient with iron deficiency anemia. The Correlation analysis between the two variables was carried out based on the normality distribution of data with an α value of 0.05. If the data distribution is normal ($\alpha > 0.05$) a Pearson correlation test is performed, if the data distribution is not normal ($\alpha < 0.05$) a Spearman correlation test is performed. This research has been approved for ethical clearance by KEPK FKUI with number: 302/UN2.F1/ETIK/PPM.00.02/2022.

RESULTS AND DISCUSSION

A total of 90 patients were diagnosed with iron deficiency anemia, consisting of 42 (46.7%) male patients and 48 (53.3%) female patients. Most of the patient data come from the teenage age. The following is a table of patient characteristics data.

Table 1. Data of patient based on age, sex and parameter

Variables	N = 90
Sex	

Male	42 (47%)
Female	48 (53%)
Total	90 (100%)
<hr/>	
*Age	
Toddler (1-5 year)	18 (20%)
Child (6-11 year)	11 (12%)
Teenager (12-25 year)	27 (30%)
Adult (26-45 year)	24 (27%)
Elderly (> 45 year)	10 (11%)
Total	90 (100%)
<hr/>	
Hb levels parameter (g/dL)	
Max	11,9
Min	4,7
Average	9,8
<hr/>	
Ret-He parameter (pg)	
Max	25,6
Min	12,4
Average	21,1
<hr/>	

*Age criteria based on the Indonesia Ministry of Health

The concentration of Hb in patients with iron deficiency anemia is 4 to 11.9 g/dL and Ret-HE values are known to be 12.4 to 25.6 pg. Both of these results are below normal values, with normal values for Hb levels of 12 to 14 g/dL and Ret-HE 29.3– 35.4 pg (Dosen TLM Indonesia, 2020)(Sysmex, 2023). The upper limit of the Ret-HE value obtained in this study was 25.6 pg which was in accordance with another study of Mehmet Ali et al (2019).

The data normality test obtained $0.000 < 0.05$, indicating that the data was not normally distributed, so the Spearman correlation test was carried out as shown in Table 1 below.

Table 2. Correlation of Ret-HE values and Hb levels in patients with iron deficiency anemia

Variabel	Mean	r	P
Ret-HE	21,12 ± 3,47	0.617	<0.001*
Hb	9,8 ± 1,69		

$P < 0.005$ = there is a correlation

$P > 0.005$ = no correlation

Spearman's correlation test results showed a value of $P = 0.000$, which means there is a correlation between Hb levels and Ret-HE values. The correlation shown in the number (r) 0.617 is in the range of 0.51 to 0.75, it shows a strong correlation between the two variables and has a

positive pattern. This shows the lower concentration of Hb, the lower the Ret-HE value which shows in Figure 1.

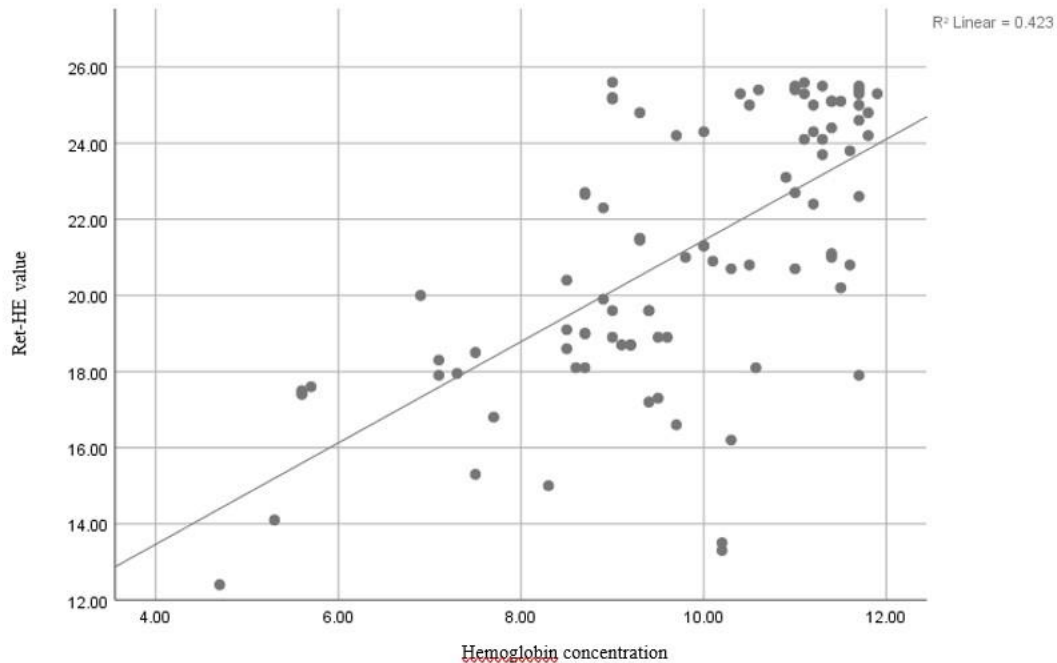


Figure 1. Correlation of Ret-HE values and hemoglobin levels

Patients with iron deficiency anemia have low concentrations of Hb due to reduced iron, which is a component of hemoglobin in erythrocytes. The gold standard for measurement of iron deficiency anemia is bone marrow histology. This method is difficult and unpracticable. The other method to detect iron deficiency anemia is ferritin which shows the iron reserves, although serum ferritin levels increase in the presence of chronic inflammation. For this reason, Ret-He which is available in a hematology analyzer can be easily obtained for screening for iron deficiency anemia (Tahara *et al.*, 2022).

Ret-He is a parameter that reflects the cellular availability of iron and it can be obtained in a short time (Sany D, El Shahawi Y, 2020). This parameter does not require additional costs because it can be measured simultaneously on a hematology analyzer, and is not affected by other chronic diseases (Uçar *et al.*, 2019). The Ret-HE value in this research was below the normal value and it was also found in the other study (Sany D, El Shahawi Y, 2020). In this study, a correlation was found between Hb and Ret-He levels and has a positive patterns (Table

2). This shows that a decrease in Ret-He levels can be in line with a decrease in Hb levels. This is also evidenced by the distribution of data in Figure 1.

Reduces Ret-He values can indicate the presence of an iron deficiency anemia in patients. The reduction in the Hb content of reticulocytes (Ret-He) in contrast to the Hb content of mature red blood cells, but these two parameters can show significant strength in identifying iron deficiency anemia status in patients (Auerbach, Staffa and Brugnara, 2021).

Measurements of Ret-He can identify iron deficiency anemia before the distinguishing changes red blood cell parameter (Uçar *et al.*, 2019). This is because reticulocyte cells are the precursors of erythrocyte cells, so the content of low Hb levels in reticulocytes can lead to low Hb levels in erythrocytes. Due to the shorter life span of reticulocytes than mature red blood cells, Ret-He measurement can identify iron deficiency before any change in other red blood cells count parameters, such as MCV. (Auerbach, Staffa and Brugnara, 2021).

CONCLUSION

There is a positive correlation between Hb levels and Ret-HE values in patients with iron deficiency anemia, this shows that the lower the Hb concentration then the lower the Ret-HE value. This suggests that Ret-HE can also be used as a supporting examination to determine iron deficiency anemia.

ACKNOWLEDGEMENT

We would like to thank the management and laboratory staff of Cipto Mangunkusumo Hospital for their cooperation during the data collection process.

REFERENCES

- Aggarwal, Alisha *et al.* (2020) 'Iron-deficiency anemia among adolescents: A global public health concern', *International Journal of Advanced Community Medicine*, 3(2), pp. 35–40. doi: 10.33545/comed.2020.v3.i2a.148.
- Andriani, V. P. (2013) *Ekuivalen hemoglobin retikulosit sebagai parameter penilaian status besi pasien penyakit ginjal kronik dengan hemodialisis*. Universitas Indonesia. Available at: <https://perpustakaan.fk.ui.ac.id/opac/index.php?p>.
- Auerbach, M., Staffa, S. J. and Brugnara, C. (2021) 'Using Reticulocyte Hemoglobin Equivalent as a Marker for Iron Deficiency and Responsiveness to Iron Therapy', *Mayo Clinic Proceedings*. Mayo Foundation for Medical Education and Research, 96(6), pp. 1510–1519. doi: 10.1016/j.mayocp.2020.10.042.

- Bain, Barbara; Bates, Imelda; Laffan, M. (2017) *Dacie and Lewis Practical Haematology*. 12th edn. Edited by Lewis. Mitchel. Elsevier Ltd.
- Dosen TLM Indonesia (2020) *Hematologi : Teknologi Laboratorium Medik*. 1st edn. Edited by E. A. Maharani. Jakarta: EGC.
- Harahap, N. R. (2018) 'Faktor- Faktor Yang Berhubungan Dengan Kejadian Anemia Pada Remaja Putri', *Nursing Arts*, 12(2), pp. 78–90. doi: 10.36741/jna.v12i2.78.
- Health research and development agency ministry of health of Indonesia (2018) 'Laporan Riskesdas 2018 Nasional.pdf', *Lembaga Penerbit Balitbangkes*.
- Pradiyadnya M, I. W. R. and Suryani, I. A. M. (2017) *Respondensi Anemia Defisiensi Besi, Fakultas Kedokteran Universitas Udayana*.
- Pramantik, D. N., Ratnaningsih, T. and Mulyono, B. (2016) 'Iron deficiency screening with content hemoglobin reticulocyte (chr) in children aged 6 months to 5 years', *Journal of the Medical Sciences (Berkala Ilmu Kedokteran)*, 47(3). doi: 10.19106/jmedsci004703201501.
- Primiastanti, P. (2013) *Nilai Ekuivalen Hemoglobin Retikulosit (Ret-HE) pada Perempuan Usia Reproduksi. Tinjauan Khusus: Sebagai Penyaring Defisiensi Besi Pada Perempuan Hamil Trimester I dan II*. Universitas Indonesia. Available at: <https://library.fk.ui.ac.id>.
- Sany D, El Shahawi Y, T. J. (2020) 'Diagnosis of iron deficiency in hemodialysis patients: Usefulness of measuring reticulocyte hemoglobin equivalent', *Saudi J Kidney Dis Transpl*, 31, pp. 1263–1272. doi: 10.4103/1319-2442.308335.
- Sysmex (2023) *Information on iron deficiency early on, Sysmex Europe*. Available at: <https://www.sysmex-europe.com/academy/library/documents/detail/information-on-iron-deficiency-early-on.html>.
- Tahara, S. *et al.* (2022) 'Clinical utility of reticulocyte hemoglobin equivalent in patients with heart failure', *Scientific Reports*. Nature Publishing Group UK, 12(1), pp. 1–8. doi: 10.1038/s41598-022-18192-x.
- Uçar, M. A. *et al.* (2019) 'The importance of RET-He in the diagnosis of iron deficiency and iron deficiency anemia and the evaluation of response to oral iron therapy', *Journal of Medical Biochemistry*, 38(4), pp. 496–502. doi: 10.2478/jomb-2018-0052.
- WHO (2011) 'Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity', *Geneva, Switzerland: World Health Organization*, pp. 1–6. doi: 2011.