THE POTENTIAL OF RETICULOCYTE HAEMOGLOBIN EQUIVALENT (RET-HE) IN IRON DEFICIENCY ANAEMIA SCREENING AT BETHESDA HOSPITAL, YOGYAKARTA

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ABSTRACT

Background: Traditionally, iron deficiency anaemia (IDA) is diagnosed with iron and ferritin status. Ret-He measurement is a promising parameter to detect iron depletion in earlier stages. Previous studies recommended Ret-He examination as IDA screening.

Objective: Compare the Ret-He level and iron status of patients with and without IDA at Bethesda Hospital, Yogyakarta.

Method: This is a cross-sectional study using laboratory record of haematological examination at Bethesda Hospital from march to August 2019. Erythrocyte indices, iron status and Ret-He measurement was further examined between IDA and non-IDA group by ANOVA, t-test or non-parametric tests.

Results: There were 105 samples, where 22 (20.95%) had complete result of Ret-He and iron status, and 10 (45.45%) diagnosed with IDA. The Ret-He level in IDA group is significantly lower than non-IDA group (17.53 ± 2.43 vs 31.50 ± 4.03 , $p < 0.001$).

Conclusion: Ret-He level is lower in IDA group, consistent with other biochemical parameters (Serum iron, Serum ferritin, TIBC, and TSAT). This finding might lead to future research on the potential of Ret-He for early detection of IDA in high-risk populations.

Keywords: Reticulocyte Haemoglobin equivalent, Ferritins, Iron, Iron-Deficiency Anaemia

INTRODUCTION

Iron deficiency anaemia, currently defined as low blood haemoglobin level with depleted serum iron, has become an important health problem in developing countries including Indonesia.^{1,2,3} Women, especially in pregnancy, has higher prevalence of IDA, which affects the foetal growth, childhood development and maternal mortality.^{4,5}

Currently, the gold standard of IDA diagnosis with Prussian blue stain of bone marrow is invasive and expensive. On that account, current clinical practise utilises indirect haematology and biochemistry parameters such as serum iron and serum ferritin. However, these parameters detect the iron depletion in the late stage; their interpretation also influenced by inflammation status.^{4,6,7} The measurement of haemoglobin content in reticulocytes (Ret-He) has been used recently to detect iron deficiency. Ret-He directly express the iron availability in earlier stage of IDA. Related to its short lifespan, Ret-He level is not affected by inflammation status and diurnal variation. Previous diagnostic studies discover different cut-offs of Ret-He.^{4,8,9} This study will examine the Ret-He profile of IDA patients at Bethesda Hospital, Yogyakarta, in order to explore its potential for early IDA detection in the future, especially in the high risk population.

METHODS

This is a cross sectional studies with medical records from Laboratory Information System (LIS) of Bethesda Hospital, Yogyakarta. We retrieved routine haematological results from March to August 2019. We included all records which fulfilled our inclusion and exclusion criteria.

The inclusion criteria are patients underwent routine

haematology examination and Ret-He measurement, irrelevant to working diagnosis pre-laboratory assessment. The patients were excluded if the medical records stated congenital cause of anaemia (i.e., thalassemia, G6PD deficiency and other congenital anaemias) and if there were more than one aetiology of anaemia found.

The clinical laboratory used Sysmex XN-1000 (Sysmex, Illinois, USA) for Ret-He analysis with flow cytometry techniques, VIDAS® Ferritin (Biomerieux, Massachusetts, USA) for ferritin assessment, and Architect ci4100 (Abbott Laboratories, Illinois, USA) to measure serum iron (SI) and total iron binding capacity (TIBC). Transferrin saturation (TSAT) was calculated manually as the ratio of serum iron (SI) to total iron binding capacity (TIBC) and expressed as a percentage ($TSAT = \frac{SI}{TIBC} \times 100\%$).

Anaemia is defined by haemoglobin level less than 11 g/dl for women and children, and less than 13 g/dl for men. Children was defined by age below 18 years old at the time of blood examination. Samples with anaemia were categorised based on mean corpuscular volume, MCV (microcytic [MCV < 80] fL, normocytic [MCV 80-100 fL]), and the diagnosis of iron deficiency anaemia (IDA). Iron deficiency anaemia was defined by low ferritin level (below the cut-off reference range by age), transferrin saturation (TSAT) below 15%, and total iron binding capacity (TIBC) over higher than the cut-off reference range.

Baseline characteristics of the samples were described by table. The association between variables and Ret-He results were analysed by independent t-test, ANOVA or Kruskal-Wallis based on the data distribution. Further post-hoc analysis was analysed to assess the

association in IDA and non-IDA subgroup. This research was reviewed and approved by the Health Research Ethic Committee at Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta (Letter No: 965/C.16/FK/2019).

RESULTS

There are 105 records from 105 patients acquired in the research period fulfilling our eligibility criteria. The subjects are consisted of (65%) male and (33%) children. Among the subjects, there are 10 without anaemia, 65 with microcytic anaemia

In the post-hoc analysis, there is no statistically significant difference of haemoglobin between microcytic anaemia group and

and 30 with normocytic anaemia. With K-S test for assumption of normal distribution, we found that haemoglobin, red blood cell count (RBC) and mean corpuscular haemoglobin concentration (MCHC) follow Normal distribution hence we used parametric analysis (ANOVA). The other variables did not follow Normal distribution, so we used nonparametric analysis (Kruskal-Wallis). Table 1 further describe the erythrocyte indices including Ret-He assessment between group (without anaemia, microcytic anaemia, and normocytic anaemia), which shows statistical significance.

normocytic anaemia group, and of MCHC between normal haemoglobin group and normocytic anaemia group.

Table 1. Erythrocyte indices between subjects without anaemia, microcytic anaemia, and normocytic anaemia at Bethesda Hospital, Yogyakarta (March-August 2019)

Erythrocyte indices	Without anaemia (N=10)	Microcytic anaemia (N=65)	Normocytic anaemia (N=30)	p-Value
Haemoglobin (g/dl) Mean ± SD	11.96 ± 0.45	7.70 ± 1.69	7.42 ± 2.13	< 0.001
RBC (10 ⁶ /μL) Mean ± SD	4.66 ± 0.33	3.97 ± 0.89	2.49 ± 0.73	< 0.001
RET-HE (pg) Median (Minimum-Maximum)	27.20 (22.50 – 30.20)	19.0 (11.70 – 32.40)	31.35 (18.00 – 36.90)	< 0.001
MCV (fL) Median (Minimum-Maximum)	76.85 (65.00 – 84.40)	62.20 (47.70 – 78.70)	87.65 (80.40 – 99.20)	< 0.001
MCH (pg) Median (Minimum-Maximum)	26.10 (22.10 – 28.50)	18.90 (12.70 – 31.30)	30.20 (24.80 – 34.40)	< 0.001
MCHC(g/dl) Mean ± SD	33.58 ± 0.88	30.75 ± 3.09	33.84 ± 1.75	< 0.001

RBC = red blood cells

Ret-He = reticulocyte haemoglobin equivalent

MCV = mean corpuscular volume

MCH = mean corpuscular haemoglobin

MCHC = mean corpuscular haemoglobin concentration

Tabel 2. Ret-He, Iron Status and Ferritin values according to iron deficiency diagnosis at Bethesda Hospital, Yogyakarta (N = 22)

Variable	Iron deficiency anaemia (N = 10)	Other than Iron deficiency anaemia (N = 12)	p-values
Ret-He (pg)	17.53 ± 2.43	31.50 ± 4.03	< 0.001
Serum iron (µg/dL)	10.33 ± 4.77	63.83 ± 63.04	0.021
TIBC (µg/dL)	530.22 ± 78.33	253.30 ± 58.14	< 0.001
TSAT (%)	1.96 ± 0.79	22.92 ± 22.85	0.014
Serum ferritin (ng/ml)	14.16 ± 11.52	633.12 ± 529.43	< 0.001

Further assessment with IDA and non-IDA group is shown in Table 2. Patients with iron deficiency anaemia have lower Ret-HE, in accordance with low serum iron, TSAT and serum ferritin and high TIBC.

DISCUSSION

Ret-He Profile

This study discovered a relatively low Ret-He in non-anaemic group (median 27.2 pg, range 22.5 to 30.2 pg) compared to the previously known reference range of 29.8 – 37.7 pg or 31,7 (28 – 36,3 pg in adults) and 23–33 pg in infants aged 9–12 months.^{10–12} The wide range of Ret-He result in this study was influenced by age and sex as confounding, which we did not adjust in further analysis. The difference of Ret-He reference range between children and adult is influenced by temporary physiological decrement of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in the first two-years of life.¹²

Ferritin Status and TIBC

This study discovered a lower borderline level of ferritin, according to cut-offs from studies.^{13,14} This finding might be explained by possible concurring inflammation, which could be caused by liver disease, alcohol consumption, hyperthyroid, aging or hormonal contraception.¹⁴ Unfortunately, this study did not explore the inflammation status of each respondents. High level of TIBC in

this study negatively correlates with the serum iron, transferrin, TSAT and Ret-He.

Potential of Ret-He to screen IDA earlier compared to other haematologic indices. The diagnosis of IDA is established with low Hb, low Ht, serum iron, TSAT and serum ferritin, and high TIBC.¹⁴ Lower Ret-He among patients with IDA is in accordance with previous studies.^{11,12} Haemoglobin within reticulocyte reflects the erythropoiesis activity in the bone marrow. The short lifetime (24 to 48 hours) of reticulocyte indicates its role in recent cause of disturbance in erythropoiesis hence important in the early detection of IDA, compared to general Hb values.¹³ Furthermore, some studies recommended ret-He for early screening of IDA with certain cut-off's (≤ 25 pg⁸, ≤ 27.5 pg¹², ≤ 26.1 pg¹⁵).

This study discovers that Ret-He might be used for early detection and diagnosis tools of IDA in the future, similar to previous studies.^{4,8,9,11,12,14,15} Ret-He measurement is low cost and rapid, using relatively small amount of specimens, and not influenced by inflammation and diurnal variation.^{8,15}

Based on literature, patients with haemoglobinopathies might also shows low level of Ret-He.¹⁶ However, this study did not further examine the haemoglobinopathies status through electrophoresis.

CONCLUSION

The Ret-He in IDA patients is significantly lower than other anaemia group in this study. These findings warrant further research on the effectivity of early IDA screening with Ret-He in clinical and public health setting, especially for high-risk population.

CONFLICT OF INTEREST AND FUNDING RESOURCES

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