INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

DIFFERENCE OF HET RE LEVEL IN THALASSEMIA B MINOR AND IRON DEFICIENCY ANEMIA

Bobby RBE Padang*[1], Bidasari Nasution[2] & Adi Koesoema Aman[1]

[1]Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara / Haji Adam Malik General Hospital, Medan, North Sumatera

[2]Department of Pediatrics, Faculty of Medicine, Universitas Sumatera Utara / Haji Adam Malik General Hospital, Medan, North Sumatera

DOI: https://doi.org/10.29121/ijrsm.v7.i11.2020.1

Keywords: Iron Deficiency Anemia, Thalassemia, Ret-He.

Abstract

Background

The most common diseases found with peripheral blood morphological features of hypochromic microcytic anemia are iron deficiency anemia and thalassemia. Hypochrome micrositer is a morphological description of red blood cells with MCV values smaller than normal (<80fl) and MCH smaller than normal values (<27pq). This morphological picture can be found in the condition of iron deficiency anemia and thalassemia. There are several markers for the assessment of Hb content in reticulocytes, including Ret-He. Ret-He, which can be measured by the latest automated hematological analysis, is considered to reflect the iron content in reticulocytes

Aim

Using Ret-He levels as a marker in distinguishing patients with β minor thalassemia and iron deficiency anemia.

Method

This research is an observational analytic study using case control measurement method, during April to July 2019 at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatra / H. Adam Malik General Hospital Medan. The population was students who were admitted to the University of North Sumatra who came for complete blood count examination. The subjects were students with MCV <80 fl, MCH <27 pq. Subjects who met the inclusion criteria then continued with the calculation of the Mentzer Index and RDW Index, ferritin serum examination using the ECLIA method, hemoglobin electrophoresis examination with micro capillary electrophoresis. By consecutive sampling, 42 samples were obtained, of which 21 subjects diagnosed with iron deficiency anemia and 21 subjects diagnosed with beta thalassemia minor were examined for their Ret-He levels using the flowsitometric method which were then analyzed statistically.

Results

From 21 iron deficiency anemia patients it was found that the average Ret-He value was 30.64 (6.08) pg and from 21 patients with beta minor thalassemia it was found that the average Ret-He value was 25.63 (6 , 72) pg. The results of the unpaired t-test for both groups obtained p = 0.016. By using the ROC curve obtained the Ret-He cut-off value in distinguishing cases of iron deficiency anemia with thalassemia was 27.30 pg with a sensitivity of 90.5% and specificity of 71.4%.

Conclusion

From the results of the study concluded that there was a significant difference between the levels of Ret-He in patients with iron deficiency anemia with thalassemia beta minor patients with a cut-off value of 27.30pg

Introduction

The most common diseases found with peripheral blood morphological features of microcytic hypochromic anemia are iron deficiency anemia and thalassemia. Hypochromic microciter is a morphological picture of red blood cells with an MCV value smaller than normal (<80fl) and an MCH smaller than normal (<27pq) .[1,2,3] Iron deficiency anemia is anemia that arises from a lack of iron supply for erythropoesis,[4,5,6], whereas Thalassemia is a group of genetic disorders that results in reduced or no synthesis of one or more globin chains[7,8,9].

Genetic disorders of thalassemia are widespread in areas in the mainland of China in the border areas of Thailand, Laos, Cambodia with a frequency of 50-60% and also spread in other parts of Southeast Asia with decreasing frequency in more distant areas. The gene frequency for Indonesia is not yet clear. . It is estimated that around 3-5%, the same as Malaysia and Singapore[7,9].

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Hemoglobin reticulocyte examination reflects a dynamic erythropoesic process in the bone marrow[10]. Ret-He is a direct index of iron availability and reflects the cellular availability of iron10. Ret-He correlates with iron deficiency and is a useful marker of iron deficiency in infants and children, adult blood donors, geriatric patients, pregnant women, and patients with chronic kidney disease undergoing hemodialysis[11,12,13]. Erythrocyte and reticulocyte indexes can be used to help differentiate thalassemic patients from iron deficiency anemia[14].

This study was conducted to differentiate levels of Ret He in thalassemia and iron deficiency anemia at RSUP H. Adam Malik Medan.

Method

This research is an analytical study with a case control design. Observations were made once to obtain data on Ret-He levels from patients with beta minor thalassemia and iron deficiency anemia at the H. Adam Malik General Hospital in Medan and the University of North Sumatra Hospital. The population is female students who are accepted at the University of North Sumatra. general check up.

Patients who meet the inclusion criteria such as 1) microcytic hypochromic (MVC < 80 fl, MCH < 27pg) 2) patients with beta minor thalassemia who have been confirmed by laboratory tests 3) iron deficiency anemia patients who have been confirmed by laboratory tests 4) maximum age 18 years 5) patients were willing to be the study sample and signed an informed consent. Subjects met the inclusion criteria followed by the calculation of the Mentzer Index and RDW Index, examination of serum ferritin using the ECLIA method, examination of hemoglobin electrophoresis with micro capillary electrophoresis. By consecutive sampling of 42 samples, 21 subjects with a diagnosis of iron deficiency anemia and 21 subjects with a diagnosis of beta thalassemia minor. Patients who had received blood transfusions in the last 3 months, received iron supplementation, and suffered from other systemic diseases were excluded from the study. this.

The EDTA venous blood sample of 42 patients was examined from April 2019 to July 2019. The examination was carried out with the Sysmex XN-1000 which had been previously carried out by quality control. Complete blood analysis was performed using automatic cell counting with the principle of flowcytometry. The data were taken in the form of complete blood and Ret He levels.

The research data analysis was performed using SPSS by performing bivariate analysis using unpaired T for data with normal distribution and the Mann Whitney test for data that were not normally distributed. The receiver-operating characteristic (ROC) curve was performed to determine the cut-off point of Ret-He levels in differentiating cases of Iron Deficiency Anemia from Beta minor Thalassemia. The p value <0.05 was said to be statistically significant

Results

Data analysis in this study was carried out on 42 patients with male gender as many as 4 people (9.5%) with the mean age of all samples 17.95 years with a standard deviation of 0.49 and the median age value is 18 years with the lowest age is 16 years and the highest age is 18 years. The characteristics of the research sample can be seen in the table below.

Table 1 Karakteristik Sampel

Variable	N	%	Mean(SD)	Median(Min-Max)
Age			17,95(0,49)	18(16-18)
Sex				
Men	4	9,5		
Women	38	90,5		
Ethnic				
Jawa	10	23,8		
Toba	9	21,4		
Melayu	8	19,0		
Mandailing	5	11,9		

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Tionghoa	3	7,1
Karo	2	4,8
Simalungun	2	4,8
Banjar	1	2,4
Nias	1	2,4
Toraja	1	2,4

Median hemoglobin (Hb) levels of patients with iron deficiency anemia and thalassemia were found to be 9.60% and 9.80% (p 0.0037). The median MCV value and mentzer index of beta thalassemia patients were lower than iron deficiency anemia (61.59; 12.30), while the median RDW value of thalassemia patients was higher (16,60). Table 2.

Table 2. Data from measurement of hemoglobin levels, RBC counts, MCV values, RDW values, Mentzer Index, RDW Index, ferritin levels in patients with iron deficiency anemia and patients with beta minor thalassemia

Variable	Iron Deficiency Anemia Mean(SD) Median(Min-Max)	Beta Minor Thalasemia Mean(SD) Median(Min-Max)	p
Hb (gr %)	9,60(8,40-10,00)	9,80(8,20-10,70)	0,037**
RBC (million/mm^3)	3,77(3,56-3,96)	5,13(4,56-5,81)	<0,001**
MCV (fL)	74,09(2,68)	61,59(4,30)	<0,001*
RDW	12,60(11,50-14,20)	16,60(11,60-18,40)	<0,001**
Mentzer Index	19,80(18,30-21,10)	12,30(9,70-12,90)	<0,001**
RDW Index	324,76(29,55)	193,80(19,64)	<0,001*
Feritin (mcg/L)	8,00(3,00-10,00)	87,00(40,00-179,00)	<0,001**

*T-Test**Mann Whitney Test

There is a significant difference between the Ret-He levels in patients with iron deficiency anemia with beta minor thalassemia patients, where the Ret-He levels in patients with Beta Minor Thalassemia (25.63 ± 6.72 pg) are lower than the Ret-He levels in patients. iron deficiency anemia (30.64 ± 6.08 pg) [p 0.016]

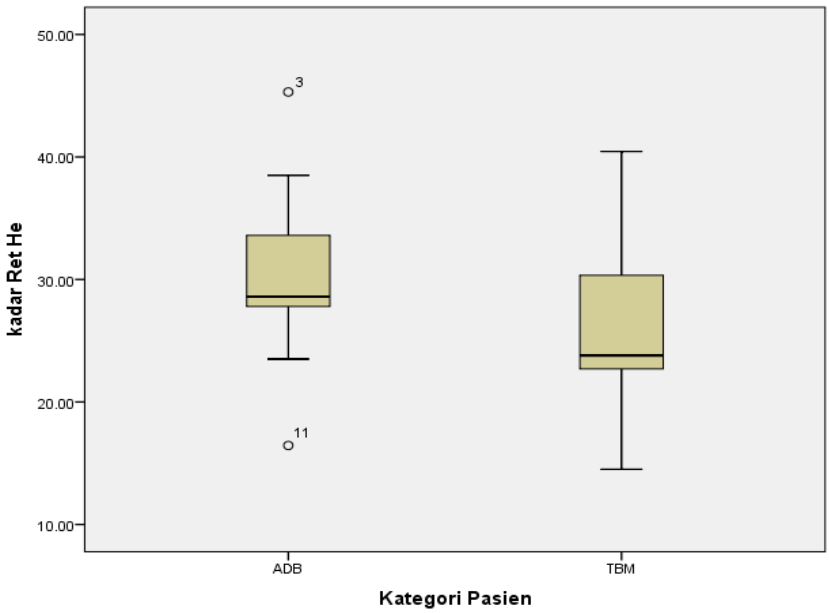

Figure 1. Comparison of Ret-He levels in patients with iron deficiency anemia with patients with beta minor thalassemia

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

The value of Ret-He discrimination in distinguishing cases of iron deficiency anemia with beta minor thalassemia was assessed by the Receiver Operating Characteristic (ROC) method and found AUC of 74.6%, with a confidence interval of 0.586 - 0.906 (95% CI, p = 0.006) with moderate quality discrimination. From this analysis, the value of the Ret-He cutoff point was 27.30 pg with a sensitivity of 90.5% and a specificity of 71.4%.

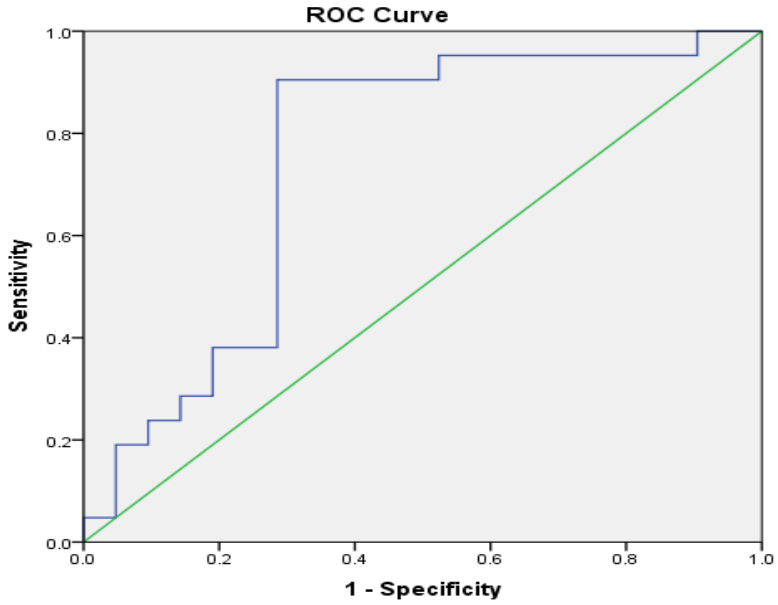

Figure 2 Receiver-operating characteristics curve analysis to assess the performance of Ret-He in distinguishing cases of iron deficiency anemia from beta minor thalassemia

Discussion

This study is a case control study which aims to determine the role of Ret-He levels in distinguishing cases of iron deficiency anemia from beta minor thalassemia. In general, iron stores are reduced or lost before people develop anemia. Therefore, erythrocyte diet and iron recycled must meet the demands for erythrocyte production. If iron loss continues, the newly produced erythrocyte lowers hemoglobin, causing the amount of iron supplied by erythrocytes to decrease. Unlike thalassemia, an increased erythrocyte count is not produced in a state of iron deficiency to compensate for the decrease in the intracellular hemoglobin level. For this reason, reticulocytosis is usually absent. In the absence of major bleeding, iron deficiency anemia generally develops slowly over months or years[15].

Thalassemia is a genetic blood disorder, which is caused when the body makes fewer healthy red blood cells and less hemoglobin than normal. Hemoglobin is the protein in red blood cells that carries oxygen to all body tissues. Hemoglobin usually consists of two α- chains and two globins. The nature of the thalassemic syndrome is associated with reduced or absent synthesis of either of these two chains. The prevalence of α-thalassemia is lower than β-thalassemia and usually does not show symptoms[16].

Most of the current screening methods for hemoglobinopathy include high-performance liquid chromatography (HPLC), hemoglobin electrophoresis, PCR mutation screening, and DNA testing. All of these methods result in higher project costs and require specialized instrumentation and trained technicians. In addition, currently various red blood cell (RBC) indexes and formulas have been designed to differentiate between thalassemia and IDA and their sensitivity and specificity are presented in various articles[16].

From the research conducted, it was found that there were significant differences between hemoglobin levels, MCV values, RDW values, Mentzer Index and ferritin levels in iron deficiency anemia patients with beta minor thalassemia patients. This is in line with previous studies that conducted research on the use of a new index for hematological parameters to differentiate cases of iron deficiency anemia from cases of beta minor thalassemia[17]. The results obtained a significant difference between hemoglobin levels, MCV values, MCH values, and ferritin

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

levels between patients with iron deficiency anemia and beta minor thalassemia patients with p values <0.001. However, there was no significant difference between the RDW values of patients with iron deficiency anemia and patients with beta minor thalassemia with a value of p = 0.9417.

From the research conducted, it was found that a significant difference between Ret-He levels in patients with iron deficiency anemia and patients with beta thalassemia minor with a value of p = 0.016, where the Ret-He levels in patients with beta thalassemia minor were lower than in patients with iron deficiency anemia. This is in line with studies regarding the efficacy of using new parameters in differentiating cases of β-thalassemia minor, iron deficiency anemia, megaloblastic anemia and aplastic anemia18. From these studies it was found that the Ret-He levels in patients with beta minor thalassemia were lower (20.92 ± 1.5 pg) compared to patients with iron deficiency anemia (21.0 ± 9.1). This is because Ret-He shows recent changes in hemoglobin synthesis in reticulocytes. The Ret He measurement is a direct assessment of the incorporation of iron in hemoglobin. This parameter is very sensitive and specific because of the short and meaningful reticulocyte life span for early diagnosis of iron deficiency erythropoiesis[18].

Reticulocyte parameters are better for detecting iron deficiency erythropoiesis than for the red cell index. Assessment of reticulocyte maturation is useful in establishing mechanisms of anemia and an effective assessment of erythropoiesis[19]. Reticulocytes are released from the marrow 18 to 36 hours before their final maturation; they provide a real-time assessment of the functional status of erythropoiesis[20].

Ret-He represents a recent change in hemoglobin synthesis in reticulocytes. The Ret He measurement is a direct assessment of the incorporation of iron in hemoglobin14. This parameter is highly sensitive and specific because of the short life span of reticulocytes and is of great significance for the early diagnosis of iron deficiency erythropoiesis[21].

The discrimination performance of Rer-He levels in differentiating cases of iron deficiency anemia is presented with an AUC value of 74.6% (95% CI = 0.586 - 0.906) which indicates moderate quality of discrimination. In this study, it was found that the Ret-He cut-off point in distinguishing cases of iron deficiency anemia with beta minor thalassemia was 27.30 pg. The results of this study indicate differences with previous studies regarding the parameters of reticulocyte hemoglobin equivalent in anemic patients

Conclusions and suggestions

There was a significant difference in Ret-He levels between patients with iron deficiency anemia and patients with beta thalassemia minor with a value of p = 0.016. The cut off value for hemoglobin reticulocytes in differentiating cases of iron deficiency anemia with beta minor thalassemia patients was 27.85 pg with a sensitivity of 90.5% and a specificity of 71.4%. A hemoglobin reticulocyte examination should be included in the complete haematological examination. It is necessary to do further research on Ret-He for its application with other diseases.

References

[1] Andrews NC. 2009. Iron deficiency and related disorders. In: Greer JP,Foerster J,Rodgers GM, Paraskevas F, Glader B, Arber DA et al,editor. Wintrobe's Clinical Hematology 12th ed. Philadelphia:Lippincott Williams and Wilkins:.p.810-34

[2] Andrews NC. 1999. Disorders of iron metabolism.New England Journal of Medicine; 341(26):1986-95.

[3] Bakta ,I Made. 2007. Anemia Hipokromik Mikrositer dengan Gangguan Metabolisme besi dalam Hematologi Klinik Ringkas ,Penerbit Buku Kedokteran EGC,Cetakan I: 26-28.

[4] Hilman RS, Kenneth AR & Henry M. 2005. Iron Deficiency Anemia in Hematology in Clinical Practice,McGraw-Hill: 1-20

[5] 5.World Health Organization, Centers for Disease Control and Prevention; 2005. Assessing the iron status of populations: report of a joint World Health Organization/Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level, Geneva, Switzerland, 6-8 April 2004. Geneva

[6] 6. Ciesla B. 2007. The Microcytic Anemias in Hematology in practice,F.A.Davis Company ,USA: 65-95.

[7] Weatherall DJ. 2006. Disorders of globin synthesis : The Thalasemias in Williams Hematology seventh edition, McGrill-Hill:633-721.

[8] Caterina Borgna Pignattinand Renzo Galanello. 2004. Thalasemias and Related Disorders in Wintrobe Clinical Hematology,11th ed, Lippincott Williams & Wilkins:1319-1365.
[9] Hoffbrand AV, Peltit J.E & Moss PAH. 2005. Anemia Hipokrom dan penimbunan besi dalam Kapita Selekta Hematologi,Edisi 4, Penerbit Buku Kedokteran EGC,Jakarta,25-27.
[10] Brugnara C, Schiller B,Moran J. 2006. Reticulocyte hemoglobin equivalent (Ret-He) and assessment of iron deficient states. Clin. Lab. Haem 2006;28: 303–8.
[11] Torsvik IK, Markestad T, Ueland PM, Nilsen RM, Midttun O, Bjørke Monsen AL. 2013. Evaluating iron status and the risk of ane- mia in young infants using erythrocyte parameters. Pediatr Res;73:214–20
[12] Semmelrock MJ, Raggam RB, Amrein K, Avian A, Schallmoser K, Lanzer G, et al. 2012. Reticulocyte hemoglobin content allows early and reliable detection of functional iron deficiency in blood donors. Clin Chim Acta;413:678–82
[13] Joosten E, Lioen P, Brusselmans C, Indevuyst C, Boeckx N. 2013. Is analysis of the reticulocyte haemoglobin equivalent a useful test for the diagnosis of iron deficiency anaemia in geriatric patients? Eur J Intern Med.;24:63–6.
[14] Urrechaga E, Borque L, Escanero JF. Erythrocyte and Reticulocyte Parameters in Iron Deficiency and Thalassemia. J Clin Lab Anal. 2011;25(3):223–8.
[15] Miller JL. Iron Deficiency Anemia: A Common and Curable. Cold Spring Harb Perspect Med. 2013;3(7):1–13.
[16] Kabootarizadeh L, Jamshidnezhad A, Koohmareh Z, Ghamchili A. Differential Diagnosis of Iron-Deficiency Anemia from β -Thalassemia Trait Using an Intelligent Model in Comparison with Discriminant Indexes. ACTA Inf Med. 2019;27(2):78–84.
[17] Jahangiri M, Rahim F, Malehi AS. Diagnostic performance of hematological discrimination indices to discriminate between βeta thalassemia trait and iron deficiency anemia and using cluster analysis : Introducing two new indices tested in Iranian population. Sci Reports Nat J. 2019;9:1–13.
[18] Thomas L, Franck S, Messinger M, Linssen J, Thome M, Thomas C. 2005. Reticulocyte hemoglobin measurement– comparison of two methods in the diagnosis of iron-restricted erythropoiesis. ClinChem Lab Med. 43(11):1193-1202. PMID: 16232085
[19] Oustamanolakis P, Koutroubakis IE, Messaritakis I, Kefalogiannis G, Niniraki M KE. Measurement of reticulocyte and red cell indices in the evaluation of anemia in inflammatory bowel disease. J Crohns Colitis. 2011;5(4):295–300.
[20] Wysocka J, Turowski D. New reticulocyte indices and their utility in hematologic diagnosis. Pol MerkurLekarski. 2000;8(49):498–502.
[21] Eguchi A, Tsuchiya K, Tsukada M, Nitta K. Clinical usefulness of reticulocyte hemoglobin equivalent (RET-He) in patients at the pre-dialysis stage and in patients on peritoneal dialysis. Nihon JinzoGakkai Shi. 2010;52(2):132–40.
[22] Toki Y, Ikuta K, Kawahara Y, Niizeki N, Kon M, Enomoto M. Reticulocyte hemoglobin equivalent as a potential marker for diagnosis of iron deficiency. Int J Hematol. 2017