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Reticulocyte Hemoglobin (Ret-He) Accuracy as A Diagnostic Parameter in Detecting Iron Deficiency Anemia in Children

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Abstrak

Hemoglobin retikulosit (Ret-He) merupakan parameter yang digunakan untuk menilai jumlah hemoglobin dalam retikulosit, dengan demikian Ret-He menggambarkan rerata distribusi kadar hemoglobin dalam retikulosit, sehingga pemeriksaan Ret-He dapat digunakan sebagai parameter untuk mendiagnosis anemia defisiensi besi secara dini. Skrining ADB yang murah dan mudah masih diperlukan. Untuk menganalisis apakah Ret-He dapat menjadi parameter dalam menentukan anemia defisiensi besi pada anak, penelitian ini merupakan penelitian uji diagnostik. Data penelitian diperoleh dari data laboratorium rawat jalan dan rawat jalan yang menunjukkan anemia mikrositer hipokromik pada bulan Juli sampai Oktober 2017 di Departemen Ilmu Kesehatan Anak RSUD Moh. Hoesin Palembang. Analisis data dilakukan dengan menggunakan perangkat lunak SPSS. Hasil penelitian dilakukan dengan membandingkan nilai Ret-He dengan nilai saturasi transferin (standar emas). Jumlah total 120 subjek terdiri dari 52 subjek dalam kelompok ADB (TfSat <15%) dan 68 subjek dalam kelompok non-ADB (TfSat ≥15%). Insiden ADB pada kelompok anemia mikrositer hipokromik di RSUD Moh. Hoesin adalah 43,3%. Nilai titik potong terbaik Ret-He adalah 21,95 pg. Berdasarkan perhitungan dengan menggunakan titik potong 21,95 pg, diperoleh data NDP 97,8%, NDN 90,5%, RKP 57,6, RKN 0,13 dan nilai AUC 99,8%. Ret-He dapat digunakan sebagai metode untuk mendeteksi ADB yang mudah dan murah.

Kata kunci: *Ret-He, Anemia Defisiensi Besi.*

Abstract

Reticulocyte hemoglobin (Ret-He) is a parameter used to assess the amount of hemoglobin in reticulocyte, thus Ret-He describes the mean of hemoglobin content distribution in the reticulocyte, so that the Ret-He examination can be used as a parameter to diagnose iron deficiency anemia early. Screening of IDA which are cheap and easy is still needed. To analyze whether Ret-He can be a parameter in determining iron deficiency anemia in children. This study is a diagnostic test study. The research data was obtained from outpatient and outpatient laboratory data that showed hypochromic microcytic anemia from July to October 2017 in Department of Pediatrics Moh. Hoesin Hospital Palembang. Data analysis was done using SPSS software. The result of research done by comparison between Ret-He value and transferin saturation value (gold standard). The total number of 120 subjects consisted of 52 subjects in IDA group (TfSat <15%) and 68 subjects in non-IDA group (TfSat ≥15%). The IDA incidence of hypochromic microcytic anemia group in Moh.Hoesin Hospital is 43.3%. The best cut off point of Ret-he is 21.95pg. Based on calculation by using Cut off point 21,95pg, the data obtained NDP 97,8%, NDN 90,5%, RKP 57,6, RKN 0,13 and AUC value 99,8%. Ret- He can be used as a method to detect ID is easy and cheap

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

INTRODUCTION

Iron deficiency anemia (IDA) is anemia caused by a lack of iron for hemoglobin synthesis. This anemia is the most common form of anemia in the world (Mz & Windiastuti, 2018). In Indonesia, according to Riskesdas 2007 incidence of anemia at age ≥ 1 year is 21.7%, infants at age 12-59 months is 28.1%, 5-14 years old is 26.4% and 15-24 years old is 18.4% .2 The prevalence of anemia in South Sumatra according to riskesdas 2007, the prevalence of microcytic hypochromic anemia is 70.1% of all anemia in children and 33.7% of them is IDA(Badan Penelitian dan Pengembangan Kesehatan, 2013).

Reticulocyte hemoglobin (Ret-He) is a parameter used to assess the amount of hemoglobin in the reticulocyte, so Ret-He describes the mean of hemoglobin content distribution in the reticulocyte and can describe the availability of iron in the erythropoesis process in bone marrow, thus is suspected to be a parameter in diagnosing IDA as well as another test in diagnosing IDA. In addition, this Ret-He examination can be performed together with complete peripheral blood tests so no additional blood samples are required (Wahtini, 2019).

Iron deficiency anemia is one of the most common health problems in children worldwide, particularly in developing countries. This condition can lead to growth and developmental disorders, cognitive decline, and weakened immune systems. Therefore, early detection of iron deficiency anemia is crucial for timely nutritional and medical

interventions. However, traditional testing methods such as hemoglobin or serum ferritin levels often have limitations, especially in detecting early stages of iron deficiency.

Reticulocyte Hemoglobin (Ret-He) is a modern hematology parameter that measures the hemoglobin content in reticulocytes, which are young red blood cells newly released from the bone marrow. Ret-He reflects the availability of iron for erythropoiesis (red blood cell formation) in real time, making it considered more sensitive in detecting iron deficiency than conventional methods. Another advantage is that the Ret-He test can be performed simultaneously with a complete blood count (CBC), is non-invasive, and provides rapid results.

Various studies have shown that Ret-He has high accuracy in detecting iron deficiency anemia, especially in children who are susceptible to this condition. Certain threshold values of Ret-He can be used as reliable diagnostic indicators to guide clinical decisions, such as iron supplementation. By integrating Ret-He parameters into routine clinical practice, healthcare providers can make earlier diagnoses and interventions, potentially improving children's overall health status and quality of life (Tantawy et al., 2020).

Although Ret-He's research has been done a lot, but research on children to find the value of Ret-He cut off point in detecting IDA is still not widely reported, therefore researchers thought it is necessary to see the diagnostic accuracy of Ret-He as a parameter in detecting iron deficiency anemia in children.

METHODOLOGY

This study is a diagnostic test study. All children who met the inclusion criteria taken as a sample. The research sample was chosen by consecutive sampling method that is all the affordable population that meets the criteria of research in Department of Pediatrics in Moh. Hoesin Hospital Palembang, by following the research flow. The study was done from July 2017 - october 2017. Inclusion criteria was all children with laboratory result showed hypochromic microcytic anemia in outpatient and inpatient unit of Moh.Hoesin Hospital Palembang. Exclusion criteria was if the parent / guardian is unwilling to be included in the research (primary data) and the laboratory medical record data was not in accordance with the research criteria (secondary data).

All subjects were examined physically, laboratory (Ret-He, SI, TIBC, transferrin Saturation, Ferritin, reticulocyte, Hb, Ht, platelets, leucocytes, erythrocytes, MCV, MCH, MCHC). Primary and secondary data analysis was performed using SPSS (*Statistical Package for Social Sciences*, Chicago, IL, USA) software for Windows. In univariate analysis, categorical data were presented in the form of frequency distributions, proportions and

percentages, while continuous data were presented as mean and standard deviation or median and range. The diagnostic value was obtained by calculating sensitivity, specificity, positive predictability, positive likelihood ratio, negative likelihood ratio and accuracy by using 2x2 table, with transferrin saturation level <15% as reference test (WHO). Ret-He cut off point and transferrin saturation and ferritin with optimum sensitivity and specificity were all obtained by receiver operating curve (RO C) method also obtained under the curve (AUC) area and by correlation test between Ret-He and transferin saturation (Notoatmodjo, 2016).

RESULT AND DISCUSSION

The first group was IDA group (TfSat <15%), which was 52 subjects and the second group was the non-IDA group (TfSat ≥15%), which was 68 subjects. The proportion of IDA incidence was 52 subjects (43.4%) and non-IDA group was 68 subjects (56.7%) of 120 subjects in hypochromic microcytic anemia group.

There was no significant difference in overall characteristics between IDA group and non-IDA group (P> 0.05). To see the characteristics of the subjects in both groups are as follows:

Table.1. Characteristics of Subject (N=120).

Characteristics	TOTAL N=120 (%)	IDA n=52(%)	Not IDA n=68(%)	*P**
Sex :				
Male	75 (62,5)	29(55,7)	46 (70,5)	0,183
Female	45 (37,5)	23(44,3)	22 (29,5)	
Age:				
< 5 year	64 (53,3)	25(48,0)	39(57,3)	0,210
5 -10 year	13 (10,8)	4(7,60)	9(13,2)	
>10 year	43 (35,8)	23(44,6)	20(29,5)	
Nutritional State:				0,728
Wellnourished	53 (44,1)	24(46,1)	29(42,6)	
Undernourished	63 (52,5)	27(51,9)	36 (52,9)	
Malnourished	4 (3,3)	1(2,0)	3(4,5)	
Group education:				
Father				0,888
Low	47 (39,1)	20(38,4)	27(39,7)	
Medium	50 (41,6)	21(40,3)	29(42,6)	
Hight	23 (19,1)	11(21,3)	12(17,7)	

Mother				0,681
Low	42 (35)	16(30,7)	26(38,2)	
Medium	62 (51,6)	29(55,7)	33(48,5)	
Hight	16 (13,3)	7(13,6)	9(13,3)	
Level Economic State				0,146
Low	51 (42,5)	26(50)	25(36,7)	
High	69 (57,5)	26(50)	43(63,3)	

**Chi-Square tests*

Table.2. Measurement of iron and peripheral blood status in the study subjects by IDA group rather than not IDA.

Characteristics	Total (n=120) Median (Min-Max)	IDA (n=52) Median (Min-Max)	Not IDA (n=68) Median (Min-Max)	*P**
Hb (g/dL)	9,20 (4,00-10,9)	8,95 (5,00-10,9)	9,40 (4,10-10,9)	0,628
SI	40,5 (5,00-230)	23,5 (7,00-62,0)	72,00 (5,00-230)	<0,001
TIBC	232 (44,0-530)	301 (117-530)	185 (44,0-481)	<0,001
TfSat (%)	24,3(2,64-94,2)	39,1 (15,0-94,2)	10,0 (2,64-14,8)	<0,001
Feritin	68,0 (10,0-8709)	18,1 (11,0-421)	125 (10,0-8709)	<0,001
Ret-He (pg)	26,8 (11,7-37,9)	20,0 (11,7-23.0)	29,7 (22-37,9)	<0,001
Retikulosit	1,25 (10,0-18,7)	1,35 (10,0-9,00)	1,20 (10,0-18,7)	0,373
Ht (%)	27,0 (11,0-42,0)	27,0 (11,0-34,0)	26,0 (18,0-42,0)	0,758
Σ WBC (/mm)	11,0 (2,10-26,8)	11,0 (2,10-20,0)	10,7 (2,10-26,8)	0,832
Σ RBC (/mm)	3,50 (1,80-7,80)	3,40 (1,80-4,76)	3,50 (1,86-7,80)	0,617
Σ Tromb (10^3/mm)	276 (30,0-1113)	276 (99,0-1113)	273 (30,0-690)	0,313
MCV(FL)	70,0 (24,0-267)	70,0 (45,5-79,4)	69,8 (24,0-267)	0,915
MCH (pg)	21,0 (12,0-67,0)	21,0 (12,0-25,0)	22,0 (13,0-67,0)	0,090
MCHC (g/dL)	32,0 (19,0-37,0)	32,0 (19,0-37,0)	32,0 (19,0-36,0)	0,531

** Mann-Whitney Test*

The measurement of iron and peripheral blood status in study subjects was using Mann-Whitney Test based on IDA group and non-IDA group. There was a significant difference between the two groups in almost all parameters P <0.05; Ret-He, SI, TIBC, TfSat and Feritin, but there was no significant differences in Hb, Reticulocyte, Ht, MCV, MCH, MCHC, number of erythrocytes, number of leukocytes, and platelet count.

The results of ROC analysis of Ret-He examination can be seen on this following curve:

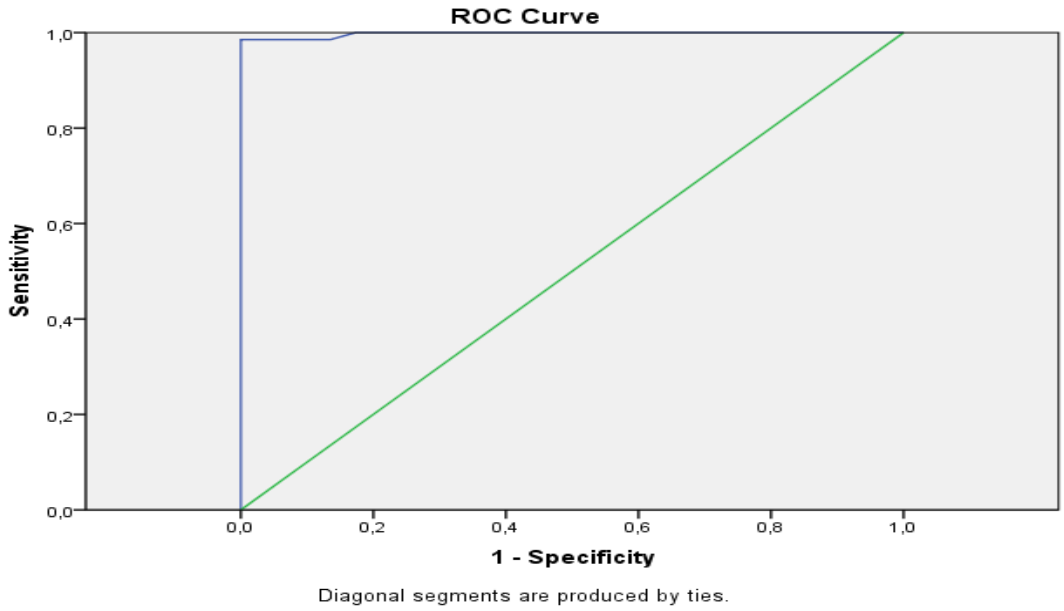

Figure 1 ROC (Receiver Operating Characteristic) Curve of Ret-He.

Table 3. *Area Under the Curve* (AUC) Ret-He.

Parameter	Area under the curve (AUC)	Standard Erorr	Confidence Interval (95%)
Ret-He	99,8%	0,002	0% - 1%

From ROC analysis above found that the area under the curve of Ret-He examination was 99,8%, (95% confidence interval 0% -1%), P <0.001. Based on ROC analysis above found that 22 pg was the best cut off point of Ret-He examination to detect iron deficiency with 100% sensitivity and 83% specificity. Based on calculation of formula by using Ret-He cut off point in this study, was obtained the Ret-He result with sensitivity 86,5%, specificity 98,5%, positive predictability 97,8%, negative predictability 90,5 %, while a positive likelihood ratio of 57.6 and a negative likelihood ratio of 0.13.

Discussion.

There was no significant relationship between general characteristics (Age, gender, nutritional status, economic status and parent's education status) and IDA incidence in children.

Proportion of IDA incidence.

The number of IDA in this study was 43.3% (52 subjects) of 120 study subjects while non-IDA was 68 subjects (56.6%), but when compared to IDA incidence according to (National Institute for Health Research & Development, 2013b) the percentage of IDA is almost as large. In Indonesia, according to Riskesdas 2007 anemia at age ≥ 1 year is 21,7%,

children at age 12-59 month is 28,1%, 5-14 years old is 26,4% and 15-24 years old is 18,4%. The prevalence of anemia in South Sumatra according to (National Institute for Health Research & Development, 2013a), 8.1% of all children are anemic and 33.7% are IDA. This high number of IDA incidence in children according to the literature is caused by multi-factors either less of iron intake, less of iron reserves or loss of iron due to specific disease.

Iron reserves of research subjects

In the measurement of iron status in study subjects, there were significant values between the two groups on almost all parameters (P <0.05); Ret-He, SI, TIBC, TfSat and Feritin, but there was no significant values found in hemoglobin and reticulocyte with P> 0,05. This may be due to some patients not having iron deficiency either based on their transferrin saturation or by other iron status parameters, but having anemia. This is similar to previous research in pregnant women (2014). The results showed significant differences in overall parameters (Ret-He, Reticulocyte, SI, TIBC, TfSat and Feritin) with (P <0.05), and hemoglobin levels value P = 0.226. This means that Ret-He can be used as an iron measurement parameter.

In this study, there was significant difference of iron content between IDA and non-IDA anemia. According to the literature, iron reserves in IDA are greatly decreased and will be followed by decreased serum iron. While in non-IDA, there are so many processes occur; one of them is metabolic disorders of iron in which iron reserves are insufficient but inadequate enough to compensate the decrease of serum iron, hence, there is a significant difference in iron levels; IDA anemia is lower than non-IDA anemia.

Validation analysis of Ret-He calculation.

In this study, Ret-He value was obtained on median of gender (Min-max): 26,8 (11,7-37,9) P-Value 0,242, Ret-He value on nutritional status 26,8 (11,7- 37,9) P = 0,244 and Ret-He value on age 26,8 (11,7-37,9) P = 0,202. This study reported that there was no significant difference in Ret-he mean based on gender, age and nutritional status. A similar study to Relaldi (2012), study on Ret-He levels in adult patients with IDA and ADC, this study reported that Ret-He had no significant differences in Ret-He mean on sex (P = 0.243) age (P = 0,254) and nutritional status with P = 0,234. Different results were reported in a study which done by Domellof. Domellof's study reported no difference in iron reserves on age and nutritional status but differences in iron reserves in the body between male infants and female infants. Domellof reported that male infants had iron reserves 40% lower than female infants with P <0.001.

The Ret-He mean in IDA group was 20.05 (11.70-23) and in non-IDA group was 29.75 (22-37.90). Differences in these both groups differed significantly with P <0.05, this can

occurs when there is an increase in iron deficiency stage, Ret-He level will decrease. According to some literatures, a significant decrease in RET-He content between IDA and CDA (non-IDA) is due to IDA serum iron levels are greatly reduced so that it directly interrupts the synthesis of hemoglobin which ultimately disrupts the erythropoesis process. Whereas in chronic disease anemia, it results from cytokines that can cause sequestration of macrophages to bind more iron, increase the destruction of erythrocytes in the spleen, suppress the production of erythropoietin by the kidneys, and cause an inadequate stimulation of erythropoesis in bone marrow, which leads to significant decrease in Ret He; iron deficiency is lower than chronic disease anemia. This is similar to study done by (Mutalazimah & Putri, 2021) in which there were also significant differences in reticulocyte hemoglobin between the iron deficiency group with non-IDA group with P = 0.001.

Based on the ROC curve, the best cut off point of Ret-He to diagnose iron deficiency and giving the best sensitivity and specificity of 100% and 83% of was 21.95pg. This cut-off value is almost similar to (Bó et al., 2023).The results of RET-He levels in adult subjects: Ret-He levels in IDA patients was 18.357 pg which were significantly different from Ret-He levels in ACD patients of 21.173 pg (P <0.05). Similar results in the (Tiwari et al., 2018) who studied IDA in adolescents, it showed that Ret-He had a high deal with CHr (93.6%) and a cut off of CHr 29 pg equivalent to Ret-He 30.5 pg with 98,4 % sensitivity and 92,2% specificity.

The results of NDP, NDN, LR + and LR- respectively 97.8%, 90.5%, 57.6 and 0.13 were similar with Ester TR study, et al (2014) ; cut off point of Ret- He 25 pg in IDA related to nutrient content, the sensitivity, specificity, NDP, NDN, LR + and LR respectively was 81.3%, 93.6%, 72%, 96%, 12.7 and 0.2. High specificity, good sensitivity and strong LR + indicated that the Ret-He value at this cut-off is suitable for the purpose of diagnosing iron deficiency anemia in children. The average cut off shows different results, iron deficiency anemia showed <30pg.

The under-the-curve (AUC) value obtained from this study showed 99.8%. That means that if the Ret-He examination is performed on 100 patients, it will provide the right conclusions in determining the presence or absence of disease in 99 patients. Based on the confidence interval, we know that the AUC of Ret-He in the population ranges from 0% - 1%. Clinically, the AUC of Ret-He is quite good because it ranges between the value of AUC recommendations expected by researcher, which is 90%. The results of this study are similar to the results of Imey's study (2013) which obtained the under-the-curve (AUC) of Ret-He in IDA patients with kidney disorders of 91.9% (95% IK 85.5% -98.3%).

Clinical Applications Ret-He in IDA.

Indonesia TfSat is used as a standard for analyzing iron deficiency anemia where a value of <15% is considered as ADB and values> 15% instead of ADB. The transferrin saturation illustrates the ratio between the existing serum iron and TIBC in percentage form so to know the value of TfSat should check for serum iron and TIBC. To obtain this TfSat additional blood sample is required because it is not included in routine blood tests but is included in clinical chemistry checks, while Ret-He's examination can be performed along with complete peripheral blood tests so it does not require additional blood samples.

SUMMARY

Ret-He can be used as a parameter to diagnose IDA with best cut off point of 21,95pg. Based on calculation by using Cut off point 21,95pg, the study got results; positive predictability 97,8%, negative predictability 90,5%, positive likelihood ratio 57.6, negative likelihood ratio 0.13 and AUC 99.8%. The proportion of IDA incidence in Moh.Hoesin Hospital Palembang for hypochromic microcytic anemia was 43.3%.

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