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Analysis of Erythrocyte Indices and Reticulocyte Hemoglobin Equivalent in Iron Deficiency Anemia on Treatment

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ABSTRACT

Assessment of treatment response is needed in the management of iron deficiency anemia (IDA). This study aims to analyze erythrocyte indices (MCH, MCV, MCHC) and Ret-He as indicators of IDA diagnosis and treatment response. A prospective cohort study in children ages 2-18 years old in orphanages throughout Makassar. Grouped into normal group and therapy group, consisting of IDA and iron deficiency groups. Elemental iron therapy 3 mg/kg/day was given. Levels of MCV (fl), MCH (pg), MCHC (g/dL), and Ret-He (pg) were measured before and on the 8th day of therapy. The normality test of numerical variable data used the Kolmogorov-Smirnov test. The statistical test used the Mann-Whitney test, Wilcoxon Signed Rank test, and the Kruskal-Wallis test. Diagnostic value and cut-off determination using ROC curve analysis. Test results were significant if $p<0.05$. The sample consisted of 40 subjects each in both normal and therapy groups. The therapy group was divided into 7 IDA and 33 iron deficiency. ROC IDA curve on MCV obtained a cut-off of 76 fl, a sensitivity of 100%, a specificity of 95%, NPP of 77.8%, NPN of 100%, MCH obtained a cut-off of 25 pg, a sensitivity of 100%, 97.5% specificity of 97.5%, NPP of 87.5%, NPN of 100%, Ret-He obtained cut-off 29 pg, sensitivity of 100%, specificity of 95%, NPP of 77.5%, NPN of 100%. MCV levels increased by 7.3% ($p<0.05$) while Ret-He increased by 19.6% ($p<0.05$) after therapy. The ROC curve coordinate on IDA showed that cut-off levels of MCV 76 fl, MCH 25 pg, and Ret-He 29 pg provided optimal sensitivity and specificity. Increasing MCV after therapy described increasing levels in erythrocyte and hematocrit synthesis. Increasing Ret-He after therapy described improving erythropoiesis quality. MCV, MCH, and Ret-He as indicators of diagnosing IDA. MCV and Ret-He monitor the success of IDA treatment response.

Keywords: Erythrocyte indices, Ret-He, IDA response

INTRODUCTION

Iron Deficiency Anemia (IDA) is the most common anemia in the world, especially in developing countries. The prevalence of IDA in children of Indonesia is still high. Riskesdas 2013 results showed 30% children of aged 1–14 years old have anemia. Prolonged iron deficiency anemia can disrupt a child's growth and development, causing defects in the immune system and brain development.[1]

Diagnosis of IDA is made based on anamnesis, physical examination, and laboratory tests. Anamnesis will find pale, weak, anorexia, irritability, deafness, and no bleeding. A physical examination will show anemic status, no organomegaly, epithelial change causing koilonychia, atrophy of the tongue papillae and change in the stomach and bowel mucosa.[1,2]

Laboratory tests that will help the diagnosis of IDA are divided into two categories, measurement that provide proof of lowered body iron and measurement that mirrors erythrocyte production that is low in iron. The changes in laboratory results caused by iron deficiency are a decrease in ferritin serum and serum iron, an increase in serum transferrin, an increase in Red cell Distribution Width (RDW), a decrease in Mean Corpuscular Volume (MCV), and a decrease in Hemoglobin (Hb). Iron deficiency consists of three stages iron depletion, iron deficiency, and iron deficiency anemia.[1,3]

Erythrocyte indices, such as MCV, Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are indicators of erythropoiesis in iron deficiency. The measurement of Ret-He can detect a change in body iron status earlier than measuring hemoglobin in erythrocytes. Body response towards iron supplementation causes an increase in reticulocyte production in 2–3 days, which reaches a peak on day 5–7.[2,4]

A 2019 study by Ucar *et al.* states that 5 days following 270 mg or oral iron therapy, does not show a significant difference in hemoglobin, but showed a significant increase of Ret-He. A study by Auerbach *et al.* in 2020, states that data analyzed from complete blood count and Ret-He can identify patients with IDA, determine the need and response of intravenous iron therapy, and decrease the time for therapeutic decisions.[5,6]

A study using erythrocyte indices (MCV, MCH, MCHC) and Ret-He in iron deficiency patients on going therapy has never been done in any population in Makassar. This study aims to analyze whether or not erythrocyte indices (MCV, MCH, MCHCH) and Ret-He can be an indicator to diagnose iron deficiency anemia and be used for monitoring the success of response towards patients with IDA ongoing iron therapy.

METHODS

This was a prospective cohort study. This study was held at several orphanages in Makassar from September 2021–October 2021. The study population was kids aged 2–18 years old at orphanages throughout Makassar that underwent screening tests consisting of clinical and laboratory examination (Hb, MCV, MCH, MCHC, Ret-He, ferritin) that fulfilled the IDA criteria by clinicians of the Pediatric Division of the Medical Faculty UNHAS. The study's sample was the population that fulfilled the inclusion and exclusion criteria. MCV, MCH, MCHC, and Ret-He were done Laboratory Installation Labuan Baji General Hospital, Makassar.

Inclusion criteria were a child living in an orphanage aged 2–18 years old diagnosed with IDA by a clinician at the Pediatric Department of the Medical Faculty of Hasanuddin University, ferritin levels < 30 ng/mL, consented to join the study and had the same therapy regimen. The exclusion criteria of this study were IDA patients that have received iron therapy, iron supplementation, or blood transfusions and IDA patients with cancer and poor nutritional status.

Iron status is based on ferritin serum. Low ferritin serum (< 30 ng/mL) is an indicator of iron deficiency.[5] The limit of anemia made by the World Health Organization (WHO) was < 11 g/dL for ages 6 months – 5 y.o, < 11.5 g/dL for ages 5–11 y.o, and < 12 g/dL for ages 12 and above. MCV (fl), MCH (pg), MCHC (g/dL), and Ret-He (pg) were measured with flow cytometry using Sysmex XN-350. Study subjects were given therapeutic hematinic that contained Ferrous Fumarate equivalent to 60 mg Fe and 0.4 mg Folic Acid, with a dosage of 3 mg/kg/day given for 7 days.

Data analysis used SPSS version 25. The normality test for numerical variable data used the Kolmogorov-Smirnov test. Statistical tests used were the Mann-Whitney test, Wilcoxon Signed Rank test, and the Kruskal-Wallis test. Determination of the cut-off value and calculation of the diagnostic value used Curve Receiver Operating Characteristics (ROC) analysis. The test results were significant if the p-value <0.05.

Each action was carried out with the consent and knowledge of the subject/guardian by signing informed consent. Ethical eligibility approval was obtained from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Makassar with recommendation for ethical approval number 611/UN4.6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

This study involved 80 study subjects, divided into two groups the normal group with 40 subjects and the group with iron therapy with 40 subjects. The group with iron therapy consisted of 7 subjects with IDA and 33 subjects with Iron Deficiency (ID) who met the inclusion and exclusion criteria (Table 1).

Table 1. General characteristics of subjects

Caracteristics	N = 80	%
Gender		
Male	40	50
Female	40	50
Age		
2-10 years old	21	26.3
11–18 years old	59	73.8
Group		
Normal group	40	50.0
Therapy group		
Iron deficiency anemia	7	8.8
Iron deficiency	33	41.3

Source: Primary data

The group receiving the therapy was divided into two, namely the IDA group with 7 subjects (8.8%) and the iron deficiency group with 33 subjects (41.3%). Meanwhile, the normal group consisted of 40 subjects (50%).

A statistically significant difference was found in all variables. Th lowest was in the IDA group with an MCV 69.8±5.5 fL, MCH 22.0±2.5 pg, MCHC 31.5±1.7 g/dL, Ret-He 23.0±4.1 pg and the highest was in the normal group with an MCV 8.9±3.2 fL, MCH

28.2±1.5pg, MCHC 34.1±0.8 g/dL, Ret-He 32.7±2.7pg ($p<0.001$).

The lowest Ret-He value in the IDA group was 27.5±6.0 pg but was not statistically significant ($p>0.05$).

According to the ROC curve (Figure 1), it can be seen that the value of the Area Under Curve (AUC) for MCV was 0.993 ($p<0.001$). This shows that the cut-off value of MCV=76.0 fl, sensitivity=100%, specificity=95%, Positive Predictive Value (NPP)=77.8%, and Negative Predictive Value (NPN)=100% could be used as a diagnostic value for IDA. MCH AUC value was 0.993 ($p<0.001$), this shows that MCH cut-off value=25.0 pg, sensitivity=100%, specificity=95%, NPP=77.8%, NPN=100% could be used as IDA diagnostic value. The MCHC AUC value was 0.873 ($p<0.01$), which indicated that the MCHC cut-off value=35.0 g/dL, sensitivity=100%, specificity=12.5%, NPP=16.7%, NPN=100% could be used as an IDA diagnostic value. The Ret-He AUC value was 0.975 ($p<0.001$), which indicated that the Ret-He cut-off value=29.0 pg, sensitivity=100%, specificity=95%, NPP=77.8%, NPN=100% could be used as a diagnostic value of IDA.

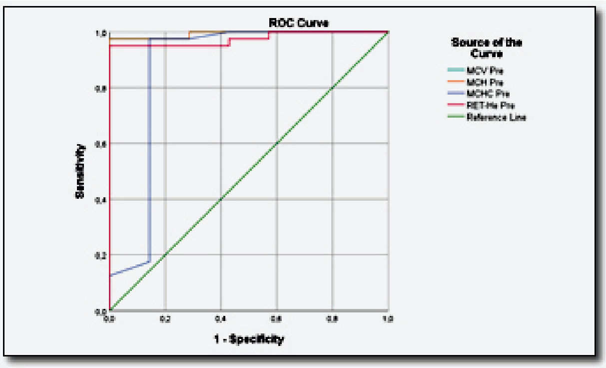

Figure 1. ROC curve for MCV, MCH, MCHC, and Ret-He towards IDA

Based on the ROC curve (Figure 2) it can be seen that the AUC MCV value was 0.740 ($p<0.001$), this indicates that the MCV cut-off value=84.0 fl, sensitivity 84.8%, specificity=37.5%, NPP=52.8%, NPN=75.0% could be used as a diagnostic value for iron deficiency. The MCH AUC value was 0.783 ($p<0.001$), which indicated that the MCH cut-off value=28.0 pg, sensitivity=81.8%, specificity=62.5%, NPP=64.3%, NPN=80.6% could be used as a diagnostic value of iron deficiency. The MCHC AUC value was 0.679 ($p<0.01$), which indicated that the MCHC cut-off value=34.6 g/dL, sensitivity=97.0%, specificity=100%, NPP=100%, NPN=97.6% could be used as a diagnostic value of iron deficiency. The Ret-He AUC value was 0.718 ($p<0.01$), which indicated that the Ret-He cut-off value=32.7 pg, sensitivity=81.8%, specificity=60.0, NPP=62.8%, NPN=80.0% could be used as a diagnostic value for iron deficiency.

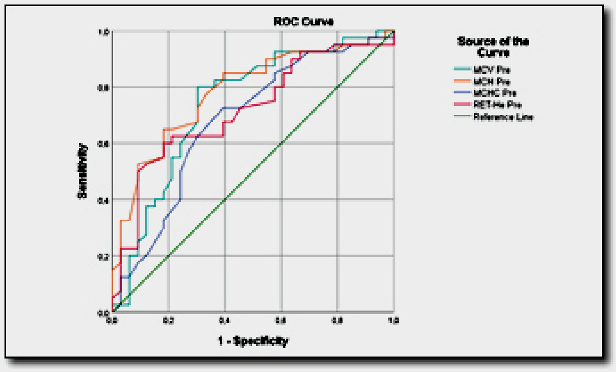

Figure 2. ROC curve MCV, MCH, MCHC, and Ret-He before and after therapy

There was a significant increase in MCV and Ret-He ($p<0.05$) before and after therapy in the IDA group. MCH increased and MCHC decreased, but both were not statistically significant ($p>0.05$).

Changes in MCV, MCH, MCHC, and Ret-He in the iron deficiency group before and after therapy showed a significant increase in MCV ($p<0.01$), MCH did not change ($p>0.05$), and MCHC decreased significantly. significant ($0<0.01$), Ret-He increased, but not statistically significant ($p<0.05$).

The age group with the most patients with iron deficiency anemia found in this study was adolescents. This is in line with a study by Triwinarni *et al.* showing anemia happens more in female adolescents compared to male adolescents, because females lose iron during menstruation, causing their need for iron intake to be higher.[7] The iron deficiency group consisted of 33 subjects (41.3%), which was greater than the IDA group, which consisted of 7 subjects (8.8%). This illustrates the need for early detection of iron deficiency events before the occurrence of anemia, to prevent long-term effects due to IDA.[1]

In the iron depletion stage, there is a progressive decrease in iron reserves without a decrease in hemoglobin and other functional iron compounds. In the iron deficient stage, iron reserves are reduced, and Hb and iron compounds begin to be limited due to decreased plasma iron transport. In iron deficiency anemia, iron reserves are greatly reduced, there is a decrease in iron transport causing the supply of iron for erythropoiesis to be reduced, and there is also a decrease in hemoglobin and hematocrit.[2] This causes the MCV, MCH, MCHC, and Ret-He in this study to be significantly the lowest in the IDA group, with MCV 69.8±5.5 fL, MCH 22.0±2.5 pg, MCHC 31.5±1.7 g/dL, Ret-He 23.0±4.1 pg and

Table 2. Comparison of MCV, MCH, MCHC and Ret-He of the group before receiving therapy and the normal group

Variable	Iron Deficiency Anemia (n=7)	Iron Deficiency (n=33)	Normal (n=40)	p*
MCV (fL)	69.8±5.5	79.8±3.9	82.9±3.2	0.000
MCH (pg)	22.0±2.5	26.8±1.4	28.2±1.5	0.000
MCHC (g/dL)	31.5±1.7	33.6±0.8	34.1±0.8	0.000
Ret-He (pg)	23.0±4.1	31.2±2.1	32.7±2.7	0.000

*Krusskal-Wallis test

Table 3. The comparison of MCV, MCH, MCHC, and Ret-He according to the group on the 8th day of therapy

Variable		Iron Deficiency(n=33)	p*
MCV (fL)	74.9±7.6	81.3±4.5	0.020
MCH (pg)	23.6±3.5	26.8±1.7	0.013
MCHC (g/dL)	31.4±2.4	33.0±1.1	0.081
Ret-He (pg)	27.5±6.0	31.6±2.3	0.126

*Mann-Whitney test

the highest in the normal group with MCV 82.9±3.2 fl, MCH 28.2±1.5 pg, MCHC 34.1±0.8 g/dL, Ret-He 32.7±2.7 pg ($p<0.001$) as depicted in Table 2.

Giving Ferrous Fumarate iron therapy increases the availability of Fe in the body. The absorption process occurs in the duodenal enterocytes. Iron is absorbed in the proximal part of the small intestine and can flow into the blood with hemoglobin, enter enterocytes, or be stored in the form of ferritin and transferrin. Iron is available in the form of ferrous ions and ferric ions. Ferric ions will enter cells through the Integrin-Mobili Ferrin (IMF) pathway, while ferrous ions enter cells with the help of Metal Divalent Transporter (DMT)-1. The iron that manages to enter the enterocytes will interact with para-ferritin to then be absorbed and used in erythropoiesis. The other part flows into the blood plasma for reutilization or is either stored in the form of ferritin or binds to transferrin.[1]

The MCV and MCH values in the IDA group were significantly lower than the ID group on the 8th day of therapy (Table 3), this illustrates that the availability of Fe in the body after iron therapy has increased and has a significant effect on MCV and MCH values in both the IDA and ID groups. This also illustrates that the daily Fe requirement in the IDA group is higher than in the ID group.[8] Ret-He in the IDA group is lower than the ID group on the 8th day of therapy, because, in IDA, iron reserves in the bone marrow decrease compared to ID, causing reduced hemoglobinization of erythrocyte precursors, which results in a lower Hb content in reticulocytes.[9]

The AUC value is a measurement of the power of certain diagnostic tests in distinguishing the presence or absence of disease in patients. In this study, the AUC MCV value was 0.993 ($p<0.001$), the AUC MCH value was 0.993 ($p<0.001$), the AUC MCHC value was 0.873 ($p<0.01$) and the AUC Ret-He value was 0.975 ($p<0.01$), all four showed significant values in calculating the diagnostic values of MCV, MCH, MCHC and Ret-He for the IDA and ID groups. MCV was found with a cut-off of 76.0 fl, a sensitivity of 100%, specificity of 95%, NPP of 77.8%, and NPN of 100%; MCH with a cut-off of 25.0 fl, a sensitivity of 100%, specificity of 97.5%, NPP of 87.5% and NPN of 100%. This illustrates that MCV and MCH can be used as effective parameters for diagnosing IDA.

Ret-He cut-off value of 29.0 pg, a sensitivity of 100%, a specificity of 95%, NPP of 77.8%, and NPN of 100% was found in the iron deficiency anemia group and Ret-He cut-off value was 32.7 pg, sensitivity of 81.8%, specificity of 60.0%, NPP of 62.8%, NPN of 80.0% in the iron deficiency group; this is in line with research conducted by Ucar *et al.*, where Ret-He was significantly lower in IDA compared to the group others (IDA 21.0±4.1 pg; ID 26.0±4.9 pg, and control 36.6±7.0 pg, $p<0.001$). In this study, Ret-He can be used as an effective parameter for diagnosing IDA.[6]

A comparison of changes in MCV, MCH, MCHC, and Ret-He before and after therapy in the IDA group found that MCV and Ret-He had a significant increase ($p<0.05$). MCV in the IDA group increased by 7.3% ($p<0.05$) while Ret-He increased by 19.6% ($p<0.05$) (Table 4). This illustrates the response to iron preparations through the absorption of iron into the body and is used in the process of erythropoiesis.[6] The MCV value provides an overview of the average size of erythrocytes. There was a significant increase in MCV before and after therapy in both groups indicating that Fe supplementation fulfilled daily Fe requirements.[8] Increased Ret-He illustrates an improvement in body iron status earlier than measuring hemoglobin content in erythrocytes, indicating the quality of the process of erythropoiesis.[2]

The MCH value increased but was not statistically significant ($p>0.05$) due to erythrocytes circulating in the blood circulation for 120 days before

Table 4. Comparison of MCV, MCH, MCHC, and Ret-He changes before and after therapy

Group	Variable	n	Mean	SD	Changes (%)	p*
Iron deficiency anemia	MCV pre	7	69.8	5.5		0.018
	MCV post	7	74.9	7.6	Increased (7.3)	
	MCH pre	7	22.0	2.5		0.089
	MCH post	7	23.6	3.5	Increased (7.3)	
	MCHC pre	7	31.5	1.7		0.236
	MCHC post	7	31.4	2.4	Decreased (0.3)	
	Ret-He pre	7	23.0	4.1		0.028
	Ret-He post	7	27.5	6.0	Increased (19.6)	
Iron deficiency	MCV pre	33	79.8	3.9		0.003
	MCV post	33	81.3	4.5	Increased (18.8)	
	MCH pre	33	26.8	1.4		0.689
	MCH post	33	26.8	1.7	No changes (0)	
	MCHC pre	33	33.6	0.8		0.002
	MCHC post	33	33.0	1.1	Decreased (1.8)	
	Ret-He pre	33	31.2	2.1		0.499
	Ret-He post	33	31.6	2.3	Increased (1.3)	

*Wilcoxon Signed Rank tests

experiencing destruction so they did not provide a significant change in the evaluation of therapeutic examinations on the 8th day in this study.[10]

MCHC was found to have decreased, but not statistically significant (p> 0.05), this illustrates a good response to therapy, namely an increase in hematocrit after administration of therapy.

CONCLUSIONS AND SUGGESTIONS

In this study, an MCV value with a cut-off of 76.0 fl, MCH with a cut-off of 25.0 fl, and Ret-He with a cut-off of 29.0 pg can be an indicators in diagnosing iron deficiency anemia. MCV levels increasing up to 7.3% (p<0.05), and Ret-He increasing to 19.6% (p<0.05) can be a monitor of sufficient response towards iron therapy for iron deficiency.

Ret-He examinations are suggested for children groups for early detection of Fe deficiency and monitoring of short-term effects of Fe supplementation.

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