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Comparative Test of Ret-He Examination in Diagnosis of Iron Deficiency in Pregnant Women

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Comparative Test of Ret-He Examination in Diagnosis of Iron Deficiency in Pregnant Women

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Abstract: The most common cause of anemia is iron deficiency, which found in 50% of anemia cases. There are 66.7% of pregnant women in Indonesia that still lack iron intake. Reticulocyte hemoglobin equivalent (Ret-He) examination as a single parameter is the initial indicator of iron deficiency, considered more sensitive in detecting iron reserves in the body. This study aims to determine the effectivity of the Ret-He examination as a single parameter in establishing the diagnosis of iron deficiency in pregnant women. This research was conducted on 30 pregnant women. The sensitivity, specificity, and predictive value of Ret-He were determined by comparing the results of the biochemical examination of iron profiles. Iron deficiency defined as an abnormal examination result on two or more biochemical parameters, which were SI <50 μg/dL, TIBC >425 μg/dL, serum ferritin <10 ng/mL and Transferrin Saturation <15.0%. Between 30 pregnant women, there were 18 (60%) with iron deficiency and 12 (40%) without iron deficiency. Ret-He levels were significantly lower in pregnant women with iron deficiency (P=0.008). A decrease in the Ret-He level in iron deficiency positively correlated with serum Ferritin (r=0.433). The effectivity of the Ret-He examination had an AUC value of 0.785 (P=0.009). Ret-He diagnostic reliability had at a cut-off value of 27.2 pg with sensitivity (87.5%), specificity (50.0%), positive predictive value (38.9%), and negative predictive value (91.7%). Ret-He examination is a useful marker of iron deficiency in pregnant women so that it can be applied for screening or as a routine examination.
Keyword: Ret-He; iron deficiency; pregnant women

INTRODUCTION

According to the World Health Organization (WHO), about 1.5 billion people or around 42% worldwide suffer from anemia (Api, Breyman, Çetiner, Demir, & Ecder, 2015). Anemia in pregnant women in Indonesia based on Riskesdas 2013 reached 37.1% (Ministry of Health Republic of Indonesia, 2013). The most common cause of anemia is iron deficiency, which found in 50% of cases of anemia. There are 66.7% of pregnant women in Indonesia that still have insufficient iron intake(Api et al., 2015; Ministry of Health Republic of Indonesia, 2013; Roosleyn, 2016). Iron deficiency anemia in pregnant women can impact on maternal death, infant mortality, congenital defects, premature birth, low birth weight babies (LBW), and anemia in babies (Roosleyn, 2016). If this does not receive individual attention, maternal and infant mortality, and morbidity in Indonesia will be challenging to reduce.

Recently, hematological examination offers a new parameter for assessing the body's iron reserves that considered to be able to replace the conventional iron

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reserves examination. Sysmex Corporation introduced the reticulocyte hemoglobin equivalent (Ret-He) (Keohane, Smith, & Walenga, 2016; Peerschke, Pessin, & Maslak, 2014). Conventional iron reserves examination (iron profile examination) has been carried out by biochemical examination that measures more than one parameter, namely serum iron (SI), total iron-binding capacity (TIBC), transferrin saturation (TSAT) and serum ferritin (Johnson Wimbley & Graham, 2011; Wu, Lesperance, & Bernstein, 2011). Ret-He examination is a single parameter that directly measures iron levels in reticulocytes, which is an early indicator of iron deficiency. This examination is considered more sensitive in detecting iron reserves in the body (Rungngu, Wahani, & Mantik, 2016; Sysmex, 2016).

Reticulocytes are precursors (young cells) of erythrocytes formed in the bone marrow and mature for two to four days in circulation. Reticulocyte examination has often used to assess the quantity of erythropoiesis. Measurement of hemoglobin level on reticulocytes can assess iron supply as an indicator of the quality of erythropoiesis. An examination of hemoglobin content on reticulocytes can detect changes in iron status much earlier than the examination of erythrocytes (Sysmex, 2016).

We aim to find the effectivity of the Ret-He examination as a single parameter in establishing the diagnosis of iron deficiency in pregnant women. Because during pregnancy, physiological changes occur in the body that can affect the results of the examination. This study focuses on diagnostic reliability by measuring the sensitivity, specificity, and predictive value of the examination.

MATERIALS AND METHOD

Patients and study design

Thirty pregnant women visited the Jagir Community Health Center (Puskesmas), Surabaya in May 2019 was taken as respondents. This study was approved by the ethics committee of the Nahdlatul Ulama University Surabaya with the Ethical Clearance Number 101/EC/KEPK/UNUSA/2019. We conducted a survey to get pregnant women with and without iron deficiency. The survey was conducted by interviewing and looking at the patient's medical records. We excluded pregnant women who were sick or had a history of chronic illness.

Venous blood sampling performed according to the phlebotomy protocol. Each respondent of pregnant women had blood drawn in the antecubital vein. The blood accommodated using a red-capped BD Vacutainer® tube (13 x 75 mm), which does not contain additives and a purple capped containing EDTA anticoagulant. Each tube filled with 3 mL of blood.

Blood on the purple tube examined for Ret-He using Sysmex XN-1000 (Sysmex Corporation Kobe, Japan). Blood in the red tube examined using the Ci8200 Architect tool (Abbott Laboratories, USA) for the serum iron (SI), total iron-binding capacity (TIBC), and serum ferritin. TSAT determined by serum iron divided by TIBC times 100%.

Iron deficiency defined as an abnormal examination result on two or more biochemical parameters that have measured. Abnormal values that were used to determine iron deficiency were SI <50 μg/dL, TIBC >425 μg/dL, serum ferritin <10 ng/mL and TSAT <15.0%. The cut-off value of examination based on the laboratory where the examination was referred to, which was the Indonesian Pramita Laboratory.

Statistical Analysis

Data were analyzed using Microsoft Excel 2013 (Microsoft, US) and SPSS version 16 (IBM, Armonk, NY, USA). A Chi-Square test used to distinguish between

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two groups. Pearson correlation test used to determine the relationship of Ret-He with biochemical parameters. Sensitivity, specificity, and predictive value of Ret-He tests determined by comparing it against the results of SI, TIBC, serum ferritin, and TSAT tests. Tests carried out using crosstab analysis in Microsoft Excel, Sensitivity, specificity, and the predictive value calculated using formulas:

$$\text{Sensitivity}\ (\%) = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}$$

$$\text{Specificity}\ (\%) = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}}$$

$$\text{Positive Predictive Value}\ (\%) = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}$$

$$\text{Negative Predictive Value}(\%) = \frac{\text{True Negative}}{\text{True Negative} + \text{False Negative}}$$

ROC curves used to determine diagnostic performance by selecting the value of the area under the curve (AUC).

RESULTS AND DISCUSSION

This study took 30 pregnant women respondents at Jagir Puskesmas, Surabaya, Indonesia. During the study, we obtained 33 respondents. Of these, three respondents did not qualify because they were sick and had a history of chronic illness. The characteristics of respondents can be seen in Table 1. The study was dominated by pregnant women aged <35 years (86.7%) with a range of 17 - 41 years, the gestational age of the third trimester of pregnancy (93.3%) with a range of 13 - 40 weeks, educated in high school and worked as a housewife.

Table 1. Characteristics of Research Respondents

Characteristic		Total	Percentage (%)
Age	<35 years	26	86.7
	≥35 years	4	13.3
Trimester	1st	1	3.3
	2nd	1	3.3
	3rd	28	93.3
Pregnancy	1st	10	33.3
	2nd	13	43.3
	3rd	4	13.3
	4th	2	6.7
	5th	1	3.3
Education	Elementary School	3	10.0
	Middle School	8	26.7
	High School	14	46.7
	Bachelor	5	16.7
Occupation	Housewife	18	60.0
	Private Employee	11	36.7
	College Student	1	3.3

The characteristics of the laboratory test results can be seen in Table 2. We found a significant difference in the examination results between the iron deficiency group and the non-iron deficiency group.

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Table 2. Characteristics of The Ret-He Examination and Biochemical Test Results in Pregnant Women

Parameter	Non-iron Deficiency		Iron Deficiency		P
	Mean	SD	Mean	SD	
Ret-He (pg)	40.0	2.3	28.0	3.1	0.008
Serum Iron (μg/dL)	90.8	45.3	75.8	92.5	0.005
TIBC (μg/dL)	360.0	55.6	466.2	42.1	0.000
Serum Ferritin (ng/mL)	33.2	38.0	10.7	7.8	0.001
TSAT (%)	24.8	9.8	15.7	17.4	0.000

Based on the results of the biochemical test, 8 (26.7%) of the pregnant women had an abnormal SI, 17 (56.7%) had an abnormal TIBC, 11 (36.7%) had an abnormal serum ferritin and 15 (50, 0%) had an abnormal TSAT. Based on the diagnosis from clinical pathologist, 18 (60%) pregnant women had iron deficiency.

Correlation between Ret-He and Iron Profile Parameters

Ret-He levels were positively correlated with serum ferritin ($r = 0.4433$; $p = 0.017$) and negatively correlated with TIBC ($r = -0.504$; $p = 0.005$). Ret-He does not correlate with Serum Iron ($r = -0.047$; $p = 0.805$) and TSAT ($r = 0.112$; $r = 0.556$). Table 3 shows the correlation of Ret-He with SI, TIBC, serum ferritin and TSAT.

Table 3. Correlations between Biochemical Tests and Ret-He

	Correlation	p-value
Serum Iron	-0.047	0.805
TIBC	-0.504	0.005
Serum Ferritin	0.433	0.017
TSAT	0.112	0.556

Effectivity of Ret-He in Diagnosing Iron Deficiency

The ROC curve shown in Figure 1 used to determine the diagnostic performance of Ret-He in iron deficiency of pregnant women. This test carried out by comparing the result of the Ret-He test to the result of the iron profile biochemical test. ROC curve analysis shows that with SI <50 μg/dL, TIBC >425 μg/dL, serum ferritin <10 ng/mL and TSAT <15.0%, the area under the curve (AUC) obtained was 0.785 (P = 0.009).

The sensitivity, specificity, and predictive value of the Ret-He examination on biochemical tests with a cut-off value of 27.2 pg are presented in Table 3 and Tabel 4 (Brugnara, Schiller, & Moran, 2006). Ret-He has a pretty good sensitivity (87.5%) and a negative forecast value (91.7%), but not with its specificity value (50.0%) and positive predictive value (38.9%).

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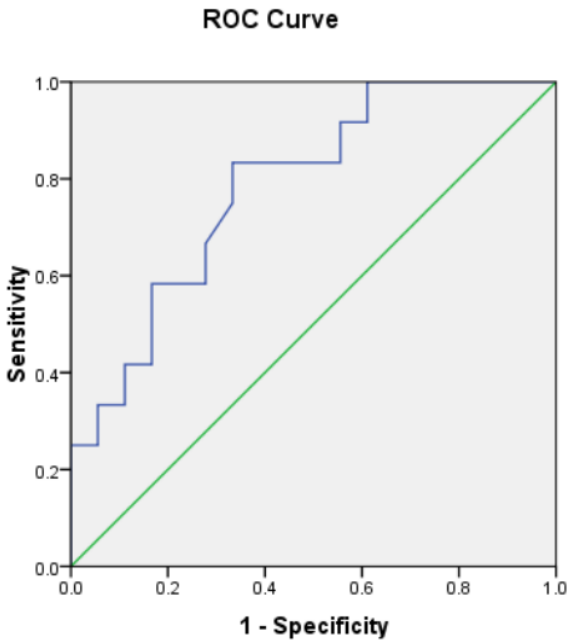

Figure 1. Analysis of The Ret-He ROC Curve in Diagnosing Iron Deficiency in Pregnant Women

Tabel 3. Crosstab Ret-He with biochemical tests

Ret-He	Biochemical Test	
	Iron Deficiency	Non-iron Deficiency
Iron Deficiency	7	11
Non-iron Deficiency	1	11

Table 4. Sensitivity, specificity and predictive value in diagnosing iron deficiency

	Result
Sensitivity (%)	87.5
Specificity (%)	50.0
Positive Predictive Value (%)	38.9
Negative Predictive Value (%)	91.7

Pregnant women are very susceptible to anemia, especially iron deficiency. This condition occurs because, during pregnancy, fetal hepcidin controls the transfer of iron from the maternal placenta to the fetal circulation. When the concentration of hepcidin is low, iron-regulated into the blood plasma and the level becomes high. When hepcidin level is high, iron transferred into enterocytes, macrophages, and hepatocytes. The need for external iron remains low, between 1 to 8 mg daily, but the need for iron increases for fetal and placental development during pregnancy (Khalafallah & Dennis, 2012; Warner & Kamran, 2017).

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Early diagnosis of iron deficiency can be made by conducting a biochemical test of the iron profile, which are serum iron (SI), total iron-binding capacity (TIBC), serum ferritin, and TSAT (Uçar et al., 2019). Various parameters of examination can have an impact on the burden of costs incurred on the patient. Therefore this study proposes a Ret-He examination in pregnant women to assess the body's iron reserves because it is faster and does not incur additional costs and can be measured simultaneously with other hematological examinations (Keohane et al., 2016; Peerschke et al., 2014).

We evaluated 30 pregnant women consisting of 18 (60%) with iron deficiency and 12 (40%) without iron deficiency. Ret-He level was significantly lower in the iron deficiency group than in the non-iron deficiency group. The decrease in the Ret-He level in iron deficiency positively correlated with serum Ferritin (r = 0.433). This means that Ret-He can be a good predictor in assessing bone marrow iron storage (Mehta et al., 2016).

The effectivity of the Ret-He examination obtained an AUC value of 0.785 (P = 0.009). Ret-He diagnostic reliability obtained at a cut-off value of 27.2 pg with sensitivity (87.5%), specificity (50.0%), positive predictive value (38.9%) and negative predictive value (91.7%). Toki et al. (2017) showed that Ret-He could be a useful clinical marker in diagnosing iron deficiency (Toki et al., 2017). Mehta et al. (2016) proved Ret-He to be an excellent marker in assessing bone marrow iron storage (Mehta et al., 2016). Ret-He can give good results in cancer patients and chronic kidney failure (Eguchi, Tsuchiya, Tsukada, & Nitta, 2010; Peerschke et al., 2014).

One of the limitations of this study is that it does not carry out the CRP examination. CRP increase as a marker of inflammation. This condition is a marker of the accuracy of serum ferritin tests because the limitation of serum ferritin is that the presence of inflammation influences it. After all, it is an acute-phase protein (Dignass, Farrag, & Stein, 2018). Also, the small number of samples and research conducted in one region contributed to the study's limitations.

CONCLUSION

Our results show that the Ret-He examination is a pretty good marker of iron deficiency in pregnant women. It can be applied for screening or can be applied as a routine examination.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest concerning this study.

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