Research Article

Identification of thalassemia carrier in anemic pregnant women at Puskesmas Kertapati Palembang: comparison of five erythrocyte indices

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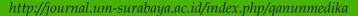3) Student of Medical Professional Study Program, Faculty of Medicine, Universitas Muhammadiyah Palembang

ABSTRACT

Anemia is a condition that is almost always found in pregnancy. Although a program of Fe tablets has been introduced during pregnancy, the prevalence of anemia during pregnancy is still high. This situation may be caused by another risk factor of anemia, such as Thalassemia. This study was aimed to investigate the presence of carrier Thalassemia in pregnant women at the Puskesmas Kertapati, Palembang, South Sumatera, using erythrocyte indices. This is a cross-sectional study with a sample size of 68 subjects, taken by purposive sampling technique. The hemoglobin level of all subjects was measured by Easy touch GCHb and confirmed by Sysmex K21. Differences in hemoglobin between Easy Touch GCHb and Sysmex K21 were analyzed by impaired t-test used SPSS program. Other data were analyzed using descriptive statistics. The mean hemoglobin levels in subjects were 10.7 g/dl. There were 68 subjects have anemia according to Easy Touch GCHb, but only 30 subjects confirmed positive anemia by Sysmex KX 21. The T-test result gave a p-value <0.05 that is considered there was a statistically significant difference between Easy Touch GCHb and Sysmex KX 21 results (p=0.001). Calculations using the England-Fraser index, Mentzer, Sirdah, and Srivastava indicate that none of the research subjects had thalassemia carrier status.

INTRODUCTION

Anemia is a circumstance that is almost always found in pregnancy. It is estimated that the prevalence reaches 18% in developed countries and 35-75% in developing countries in 2017 (Öztürk *et al.*, 2017). In Indonesia, the proportion of Thalassemia carriers among blood donors in Banyumas was 8.74% (Hapsari and Rujito, 2015). Anemia in pregnancy is a physiological condition due to an increase in maternal blood volume. This condition is called hemodilution. If left unhindered, anemia in pregnancy is associated with increased morbidity and mortality for both mother and baby (Stephen *et al.*, 2018).

The most common cause of anemia in pregnancy is iron deficiency anemia (IDA) because there is an increasing need for iron throughout pregnancy (Alene and Dohe, 2014). Until this research report was made, there was no global data on the prevalence of anemia in pregnancy, but several reports showed a prevalence of 18-46.2% (Suega *et al.*, 2002; Sukrat *et al.*, 2010; Suryanarayana *et al.*, 2017; Srour *et al.*, 2018; Stephen *et al.*, 2018). There are no national data in Indonesia about the prevalence of this disease. Several studies conducted in various regions in Indonesia in the last five years showed a prevalence between 26.7-42.6% (Astriana, 2017; Astuti and Kulsum, 2018).

The high prevalence rate of anemia in pregnancy raises a question because the Ministry of Health of the Republic of Indonesia has established a program of giving Fe tablets to all pregnant women who come to health facilities during antenatal care. Therefore, other causes of anemia in pregnancy need to be considered, such as infection, nutritional deficiencies other than iron, and Thalassemia.

Thalassemia is a genetic disorder characterized by abnormalities in erythrocytes, precisely in the globin chains' arrangement. The disorder causes the erythrocytes to undergo lysis, resulting in anemia (Viprakasit and Ekwattanakit, 2018). This disease is inherited from carrier parents to their children through the autosomal recessive pathway (Angastiniotis and Lobitz, 2019). Thus, the diagnosis of carrier Thalassemia is important to prevent the inheritance of the children. However, the definitive diagnosis of Thalassemia or the carrier requires enormous costs, so various calculations are used, which will increase the sensitivity and specificity of one another when used together. Some calculations that can be used to establish a carrier diagnosis of Thalassemia are England-Fraser index (MCV-RBC-5xHb-3.4), Mentzer index (MCV/RBC), Sirachainan index (1.5xHb-0.05MCV), Sirdah index (MCV-RBC-3xHb), and Srivastava index (MCV/RBC). This study was aimed to investigate the incidence of carrier Thalassemia in pregnant women at Puskesmas Kertapati, Palembang, South Sumatera, Indonesia, using erythrocyte indices. Erythrocyte indices are used to differentiate iron deficiency anemia or thalassemia carrier which causes anemia in a person.
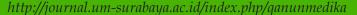
METHODS

This study was a cross-sectional study. The sample size of 68 subjects was acquired by purposive sampling. Inclusion criteria for this study were pregnant women with anemia and pregnant women undertaking antenatal care and consumed Fe tablets routinely at Puskesmas Kertapati, Palembang, South Sumatera during October-December 2019. This study's exclusion criteria were pregnant women who were experiencing bleeding in the digestive tract, urinary tract, or respiratory tract.

Determination of anemia status (Hb $\leq$ 11 g/dL) was done in two ways. Step one, the hemoglobin (Hb) level of pregnant women, was examined using the Easy Touch GCHb (Bioptik Technology Inc., Taiwan) at Puskesmas

Kertapati. Easy Touch is an automated blood analyzer that gives a quick result, and at a relatively low price, this tool is very widely used in first-level health facilities. In step two, the Hb level, red blood cells (RBC), and Mean Corpuscular Volume (MCV) of pregnant women who had anemia, according to Easy Touch GCHb, were examined using Sysmex KX 21 (Sysmex Corporation, Japan) at Palembang Health Laboratory Center. Sysmex KX 21 is an automated hematology analyzer, a standard diagnostic tool for hematology analysis. The erythrocyte index used was limited to 5 indices calculated using the Hb, MCV, and RBC values. The Ethical Committee of Faculty of Medicine Universitas Muhammadiyah Palembang approved this study with certificate No. 51/EC/KBHKI/FK-UMP/XI/2019.

All statistical data were analyzed by the SPSS program. Unpaired t-test was applied with α value 95% so p-value less than 0.05 considered as statistically significant. The mean value of Hb level, RBC, MCV, and anemia distribution based on pregnancy was analyzed using descriptive statistic analysis.

RESULTS

The results of Hb measurement using Easy Touch GCHb showed that the mean Hb of this study was 9.37 g/dL, and the majority had mild anemia (82.4%). The Hb measurement results using Sysmex KX 21 showed that the mean Hb of this study was 10.79 gr/dL. The t-test result showed a p-value <0.05, so there was a significant difference between Easy Touch GCHb and Sysmex KX 21. This result is summarized in table 1.

Table 1. Mean of Hemoglobin using two measurements (n=68 subjects)

	Easy Touch	**Sysmex**	***p*-value**
Mean Hb in g/dL (SD)	9.37 (1.23)	10.79 (1.66)	0.001
Mean MCV	-	95.16	-
Mean RBC	-	3.64	-

The results of Hb measurement using Easy Touch GCHb showed in Table 2 that the majority of respondents who had anemia were in trimester III (25 peoples or 36.76%), while according to the results of the Sysmex, the majority were in trimester II (18 peoples or 26.47%).

Table 2. Distribution of anemia based on pregnancy trimester (n=68 subjects)

Trimester	**Easy Touch**	**Sysmex K21**
I	23 (33.8%)	11 (16.2%)
II	20 (29.4%)	18 (26.5%)
III	25 (36.8%)	9 (13.2%)

Table 3. Erythrocyte indices result (n=68 subjects)

Index	Cut-off point	Iron Deficiency Anemia	Thalassemia carrier
England-Fraser (MCV-RBC-5xHb-3.4)	TC <0 IDA ≥0	68 (100.0%)	0 (0.0%)
Mentzer (MCV/RBC)	TC <13 IDA ≥13	68 (100.0%)	0 (0.0%)
Sirdah (MCV-RBC-3xHb)	TC <27 IDA≥27	68 (100.0%)	0 (0.0%)
Srivastava (MCV/RBC)	TC <3.8 IDA≥3.8	68 (100.0%)	0 (0.0%)
Sirachainan (1.5xHb-0.05MCV)	TC <14 IDA≥14	58 (85.3%)	10 (14.7%)

DISCUSSION

The mean hemoglobin levels in subjects were 10.7 g/dl. This value was similar to the mean hemoglobin levels of Cameroon pregnant women but lower than pregnant women in Northwest Morocco who have a mean hemoglobin level of 11.80 g/dl (Kimbi *et al.*, 2017; Bakrim *et al.*, 2018). Hemoglobin in pregnancy will decline during the first trimester and going further until the second trimester. Nevertheless, during the third trimester, the hemoglobin will rise (Li *et al.*, 2017). This study found the result was similar to that theory because the lowest number of anemic pregnant women was in the trimester 3 group (13.2%), and the most significant number was in the trimester 2 group (26.5%). Anemia in pregnancy is considered as one of the major risk factors for contributing 20-40% of maternal deaths directly or indirectly through cardiac failure, preeclampsia, antepartum hemorrhage, postpartum hemorrhage, and puerperal sepsis. It is also responsible for low birth weight, which in turn might contribute to an increased percentage of infant mortality (Prakash and Yadav, 2015).
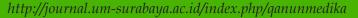
This study used two hemoglobin measurements to determine anemia status in research subjects, the Easy Touch GCHb and the Sysmex KX 21. This study indicated that 30 subjects did not have anemia according to the Sysmex KX 21 but classified as anemia according to the Easy Touch GCHb. Statistical analysis showed a statistically significant difference in mean Hb between these two instruments (p=0.001). Easy Touch GCHb measures the hemoglobin level from capillary blood, while Sysmex KX 21 measures hemoglobin level from venous blood. This occurs because a drop of capillary blood reflects the capillaries, arterioles, and venules, while venous blood reflects blood vessels, hearts, and arteries. Examination using Sysmex, which has the way flow cytometry, certainly has the advantage of getting a high accuracy hemoglobin value. While the examination of hemoglobin using Easy Touch GCHb works by measuring two wavelengths, namely 565 and 880 nm (Patel *et al.*, 2013).

This study also showed that the majority of pregnant women with anemia were in the third trimester (36.76%) according to Easy Touch GCHb, but according to Sysmex KX 21 measurement, the majority of pregnant women with anemia were in the second trimester (26.47%). These results are generally the same as various existing theories, namely anemia in pregnancy generally occurs in trimesters II and III. This is due at that time because of an increase in maternal blood plasma volume up

to 50%, resulting in hemodilution. Besides, iron demand also increases because the need for fetal erythrocyte production will increase rapidly in the second trimester. Without adequate iron intake, iron needs will be difficult to fulfill, thus disrupting erythrocytes production (Shagana *et al.*, 2018). However, anemia can occur every trimester in pregnancy (Paendong, Suparman, and Tendean, 2016; Amini, Pamungkas, and Harahap, 2018).

Calculations using the England-Fraser index, Mentzer, Sirdah, and Srivastava indicate that none of the research subjects had thalassemia carrier status. Meanwhile, calculation using the Sirachainan index indicated that there were 10 Thalassemia carriers (14.7%). The Mentzer index has a sensitivity of 89.01% and a specificity of 78.65%, ranked 3rd in terms of the accuracy of the 28 indexes used in his study. The Sirdah index has a sensitivity of 80.26% and a specificity of 88.65%, ranked 5th. The Srivastava index has a sensitivity of 74.86% and specificity of 80.81%, ranked 16th. The England-Fraser index has a sensitivity of 62.94% and specificity of 85.41%, ranked 19th. And, the Sirachainan had a sensitivity of 35.94% and specificity of 74.86%, ranked 25th. Sirachainan index has the lowest sensitivity among five erythrocyte indices (Jahangiri, Rahim and Malehi, 2019). Low sensitivity means that the index is not capable enough to classify pregnant women as a Thalassemia carrier (Parikh *et al.*, 2008).

Thalassemia is a genetic disease with anemia as the dominant clinical manifestation. An autosomal recessive pattern inherits this disease. The autosomal recessive disease will need two alleles to manifest. People with one allele of mutation for Thalassemia will not manifest the symptoms. This people are called carriers. Both Thalassemia type, alpha and beta, have the carrier. This study did not go through a standard diagnostic tool, which is Hemoglobin electrophoresis, because the screening test

found no one as a Thalassemia carrier. Suppose there is a subject with a positive result for thalassemia carrier in this study. In that case, the subject's blood will be sent to the laboratory for hemoglobin electrophoresis examination and determined what type of Thalassemia do the carriers have.

Based on the accuracy of the five indices used in this study, the Sirachainan index has the weakest accuracy. The sensitivity value is <50%, which means that the Sirachainan index is not suitable for screening a Thalassemia carrier. Thus, the difference in results on calculations using the Sirachainan index can be ignored because other indices' sensitivity was more than 50%.

CONCLUSION

There was no Thalassemia carrier in anemic pregnant women at Puskesmas Kertapati, Palembang, South Sumatera based on England-Fraser index, Mentzer index, Sirdah, and Srivastava index.

ACKNOWLEDGEMENT

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