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Early Detection Of Thalassemia Carrier In Patients With Systemic Lupus Erythematous: The Use of Shine and Lal Index

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A B S T R A C T

Background: Anemia in systemic lupus erythematous (SLE) is common due to impaired erythropoietin response and the presence of antibodies that attack erythropoietin. Various anemia in SLE include autoimmune hemolytic anemia (AIHA), anemia of chronic disease (ACD), or iron deficiency anemia (IDA). Interestingly, Indonesia lies in thalassemia belt area and it is estimated to harboring around 6-10% thalassemia carrier in its population. Therefore, anemia in SLE patients should be further examined for its possibility for thalassemia carrier. This study aimed to determine whether a simple erythrocyte index could be used for thalassemia carrier early detection of SLE patients living in Indonesia. **Methods:** This study had a retrospective and cross-sectional design, collecting hematology data of SLE patients registered at Dr. Hasan Sadikin General Hospital. Erythrocyte indices, including Mentzer index (MCV/RBC) and Shine & Lal index (MCV.MCH.MCH/100), were assessed to determine IDA or thalassemia carrier. **Results:** Of 259 hematology data from SLE patients included, predominantly female (95.8%), aged 34 years old (median; range 17-65 years) and single (27%), of whom 45.2% had anemia. However, most of them were anemia normocytic or normochromic. Interestingly, Mentzer index (<13) was found in 2.7% and Shine & Lal index (<1530) in 10% patients, suggesting consider thalassemia carrier. **Conclusion:** Although Shine & Lal index has lower sensitivity compared with Mentzer index, Shine & Lal Index might serve a broader screening tool as an early detection for thalassemia carrier.

1. Introduction

Systemic lupus erythematous (SLE) is a chronic autoimmune inflammatory disease, that has a wide distribution of clinical features and a variety of disease course features.[1,2] There are no specific disease manifestations that can describe SLE disease activity, however, hematological disorder is common with anemia, which present in about 50% of patients.[3,4] The most frequent etiology of anemia in SLE include anemia of chronic disease (ACD), iron deficiency anemia (IDA), and autoimmune hemolytic anemia (AIHA). Impaired erythropoietin response and the presence of antibodies

attacking erythropoietin contribute to the pathogenesis of anemia.[4] Furthermore, thrombocytopenia may occur, and this phenomenon is associated with antiphospholipid antibody syndrome, involving auto-antibodies against the platelets and glycoprotein IIb/IIIa or thrombopoietin receptor.

Interestingly, Indonesia is a country that falls within the scope of Thalassemia Belt. It is estimated that around 6 to 10% of the Indonesian population is carrying α or β-globin gene mutation, known as thalassemia minor or trait or carrier.[5,6] Thalassemia minor is inherited by autosomal recessive mode.[7,8] Therefore, an early detection of thalassemia carrier is needed for a better family plan.[9] Individuals with thalassemia carrier typically have an asymptomatic and/or mild anemia. Determining the cause of anemia is important because IDA and thalassemia carrier have almost the same characteristics but have different ways of treatment. Early detection of thalassemia carrier can be considered to determine the problem of hereditary disease before marriage.[9,10]

The red blood cell index can be used for early detection using various erythrocytes formula,[11,12] among others Mentzer index as well as Shine & Lal index. Mentzer index has been reported to be the most reliable index with the highest sensitivity (98.7%) and specificity (82.3%),[13] whereas the Shine & Lal index has a very high specificity up to 100%, however, the sensitivity value is low.[14]

The most prevalent cause of anemia in Indonesia is iron deficiency, and other cause of anemia is underrecognized. As for anemia in SLE patients living in Indonesia, there is a possibility that those SLE patients might also carry thalassemia gene mutation. Interestingly, the prevalence of thalassemia carrier in SLE patients is considered low, however, when the two conditions co-exist, it may result in worse disease manifestations.[15] Therefore, this study aimed to explore the distribution of thalassemia carrier among SLE patients. Furthermore, the role of erythrocyte indices Mentzer and Shine & Lal were assessed to distinguish IDA and thalassemia carrier, thus to be used for early detection.

2. Methods

This was a descriptive study with a retrospective, cross-sectional design. The latest hematology data of SLE patients registered in the Hasan Sadikin Lupus Registry was retrieved from January to November 2020. Only those younger than 65 years old were included to minimize the cause of other factors involved in anemia, such as aging. The incomplete, missing, or inaccessible hematological data was excluded. The ethical clearance was exempted and granted by the Research Ethics Committee, Universitas Padjadjaran no. 719/UN6.KEP/EC/2020 and no. 120/UN6.KEP/EC/2022.

Anemia status was designated as hemoglobin (Hb) level lower than 13 g/dL for male and lower than 12 g/dL for female. The MCV was grouped into low MCV (microcytic; <80 fL), normal (normocytic 80-96 fL), and above normal (>96 fL). The MCH was classified into low (hyphochromic; <27.5 pg), normal (normochromic, 27.5-33.2 pg), and above normal (>33.2 pg).

Mentzer index was designated as MCV/RBC and Shine & Lal index as MCV*MCH*MCH/100, with cut off value 13 and 1530, respectively.16,17,18 Value above cut off was determined as IDA and under as thalassemia carrier.

Platelet value was further grouped based on the normal range 150 to 400 x103/mL; and for leukocyte value was in the range of 4.5 to 11.0 x 109 WBCs/mL.

Data were analyzed using IBM SPSS statistics v.16 and presented as frequency in tables.

3. Results

Hematology data of 259 SLE patients was collected, with predominantly female (95.8%), aged 34 years old (median; range 17-65 years). Of note, there was 27% unmarried patients with median age of 24 years old (range 17-48 years) as shown in **table 1**. Anemia, designated as low Hb based on gender, was detected in 45.2% (117 of 259) SLE patients. Among female (n248), the proportion of anemia was 46.7%, whereas among male (n11) anemia was only found in 9.1%.

Table 1. The characteristics of Systemic Lupus Erythematosus patients registered in the Lupus Registry Dr. Hasan Sadikin General Hospital 2020

	Total	**Anemia Status***	
		Anemia	**Not Anemia**
	(n 259)	**(n 117)**	**(n 142)**
Gender			
Female	248 (95.8%)	116 (46.7%)	132 (53.2%)
Male	11 (4.2%)	1 (9.1%)	10 (90.9%)
Age			
Median (min-max)	34 (17-65)	32 (17-65)	35 (17-64)
Marital Status			
Single	70 (27%)	39 (33.3%)	31 (21.8%)
Married	182 (70.3%)	76 (65%)	106 (74.6%)
Divorce	7 (2.7%)	2 (1.7%)	5 (3.5%)

Note. *Anemia status based on low hemoglobin level for male <13 g/dL and for female <12 g/dL

MCV and MCH value were further analysed among those with anemia, and classified as having low, normal, and high MCV and/or MCH. The distribution of MCV and MCH was most often normal, both in those with anemia and not anemia SLE patients. However, there were 12.4% (32 of 259) SLE patients with anemia and 3.4% (9 of 259) without anemia who also had low MCV and/or MCH (**table 2**). Furthermore, platelet and leukocyte value were also classified into low, normal, and high and most of the patients had normal value.

Table 2. The distribution of anemia status among patients with Systemic Lupus Erythematosus based on MCV and MCH values

	Anemia Status*	
	Anemia (n 117)	Not Anemia (n 142)
MCV and/or MCH		
Low	32 (27.3%)**	9 (6.3%)**
Normal	72 (61.5%)	126 (88.7%)
High	13 (11.1%)	7 (4.9%)
Thrombocytes		
Low	12 (10.2%)	6 (4.2%)
Normal	94 (80.3%)	129 (90.8%)
High	11 (9.4%)	7 (4.9%)
Leukocytes		
Low	35 (29.9%)	22 (15.4%)
Normal	73 (62.3%)	102 (71.8%)
High	9 (7.6%)	18 (12.6%)

Note. *Anemia status based on low hemoglobin level (<13g/dL for male and <12g/dL for female). Normal range of MCV (80-96fL), MCH (27.5-33.2pg), Thrombocyte (150-450 x10^3 /ul) and Leukocyte (5-10 x10^3 /mm). **SLE patients with anemia and not anemia with low MCV and/or MCH and need further examination

To determine the etiology of anemia, Mentzer index and Shine & Lal index were calculated whether SLE patients were considered to be iron deficiency anemia or being carrier thalassemia. Based on Mentzer index, iron deficiency anemia was detected 13.1% whereas 2.7% were considered to have carrier thalassemia. In contrast with Shine & Lal index, those with low MCV and/or MCH were considered to be carrier thalassemia in 10% whereas iron deficiency anemia was 5.8% as shown in **table 3**.

Table 3. Differentiation of iron deficiency anemia and consider thalassemia carrier among Systemic Lupus Erythematosus with low MCV or/and MCH (n=32)

Erythrocyte Index	Low Hb	Normal Hb
	low MCV and/or MCH n=32	low MCV and/or MCH n=9
Mentzer		
<13	5 (1.9%)*	2 (0.8%)**
≥13	27 (10.4%)***	7 (2.7%)***
Shine & Lal		
<1530	22 (8.5%)*	4 (1.5%)**
≥1530	10 (3.9%)***	5 (1.9%)***

Note. * consider carrier thalassemia; ** consider other hemoglobinopathy; *** iron deficiency anemia

4. Discussion

Anemia, leukopenia, and thrombocytopenia are the major hematological manifestations in SLE patients,[3] being anemia is the most prevalent. The cause of anemia in SLE include autoimmune hemolytic anemia (AIHA), anemia of chronic disease (ACD), and iron deficiency anemia (IDA).[3] Iron deficiency anemia is common as a result of gastrointestinal blood loss and menorrhagia.[19,20] Furthermore, defects in the immune system in SLE may cause specific red blood cells (RBC) autoantibodies that lyse RBC. Antibody-induced damage to blood cells by complementary or separate pathways has long been considered as a common pathogenic process for SLE cytopenias.[20] Moreover, active immune or inflammatory response may reduce iron uptake at varying sites, which subsequently lead to ACD.

The prevalence of low platelets and/or low leukocytes is higher in SLE patients with anemia than SLE patients without anemia. Low thrombocytes may occur by various mechanisms including impaired production of platelets in the bone marrow, platelets sequestration in the spleen or accelerated platelets destruction in the peripheral circulation.[22] Low leukocytes count might be due to disease activity, immunosuppressive therapy, or the

presence of a virus. Furthermore, the deficiency of CD55 and CD59 surface expressions of complement regulatory proteins has been linked to decreased leukocytes in SLE patients.[3]

In our study, 45.2% of SLE patients has anemia. Furthermore, SLE patients with low MCV and MCH values (27.4%) need to differentiate for the cause of IDA or thalassemia carrier. Anemia in SLE with normocytic or normochromic characteristics is mostly caused by ACD.[3] In our study, the Mentzer index and Shine & Lal index had been calculated among SLE patients with anemia who had low MCV, resulting in 2.7% (n 7) and 10% (n26) patients with low Mentzer index and Shine & Lal index, respectively, considering thalassemia carrier among SLE patients.

Since Indonesia is located in thalassemia belt and the SLE patients are predominantly female, further exploration to thalassemia carrier is needed. The age of SLE patients in our study is ranging from 17-65 years (median 34 years old) and 27% is still single or not married, showing that many patients are still in productive age. At this stage, it is important to provide education and early detection of thalassemia carrier for better family planning. Premarital screening or even earlier period in adolescence need to be emphasized.[22]

Furthermore, it has been shown, that when the SLE and thalassemia carrier co-exist, the SLE manifestations might occur in more severe disease activity. This might associate due to beta-globin gene in chromosome 11 is close to various genes that have prominent roles in immune regulation.[23] Reduced expression of hemorphin, an endogenous opioid peptides derived from hemoglobin, may also play a role in severity of lupus.[23] Since the criteria of disease activity is not representable in our study, active disease status of SLE patients whether patient is in remission state or flare state has not been assessed, that limits our study.

Hb analysis and molecular examination facilities in our study are limited. Recently, early detection of thalassemia carrier has been possible in simply way by using erythrocyte index,[13] which is rapid and cost-effective. Low MCV and/or MCH alone are useful predictor in a resource limited area to early screen thalassemia carrier.[24] Various methods for erythrocyte indices formula have been proven to have a fairly high level of sensitivity and specificity based on the results of previous studies.[16,17,18] The concordance test of Shine & Lal, even with limitation in sensitivity, has shown a good specificity that might help in area with limited laboratory equipment.[25]

Interestingly, the reticulocyte hemoglobin equivalent (RET-He) can also be used to detect the etiology of anemia more effectively. RET-He is capable to detect iron availability used in erythropoiesis process. The RET-He parameter has a sensitivity of 93.3% and a specificity of 83.2% to distinguish iron deficiency anemia.[21] However, on blood tests 8 parameters in our study, RET-He data are not available. Therefore, simple erythrocyte indices such as Mentzer index or Shine & Lal index might of great use, especially in area with limited resources.

The Mentzer index is used as a reference for this examination because this index has the highest reliability value with HB analysis.[16] In the other end, the Shine & Lal index is used because it has high specificity. Interestingly, our data shows that of 117 SLE patients with anemia, 12.4% have low MCV and MCV. Mentzer index results in 2.7% considered thalassemia carrier whereas Shine & Lal index in 10%. Therefore, further diagnosis of thalassemia carrier can be made after Hb electrophoresis examination.[11] However, a definite diagnosis is needed to confirm the thalassemia carrier by using DNA examination. Moreover, DNA analysis is pivotal in identifying the possible existence of alpha thalassemia carrier.

This study has some limitations. The hematology data available is limited to only 8 parameters, and no reticulocyte hemoglobin equivalent (RET-He) data is available to support the anemia cause in SLE. Furthermore, Hb-electrophoresis is not performed. Here, the thalassemia carrier can only be determined based on erythrocyte indices. False negative or positive can be thus occurred, which may have implication in the future. However, the erythrocyte indices may help to identify consider thalassemia carrier among SLE patients and therefore, referring the SLE patients for further Hb-electophoresis examination and DNA test to confirm is highly advised.

5. Conclusion

Thalassemia carriers among SLE patients based on

Shine & Lal index is 10%, similar to the prevalence of thalassemia carriers in general population in Indonesia, a country in global thalassemia belt. Therefore, SLE patients with low Shine & Lal Index should be referred for further examination to explore the etiology of anemia in SLE.

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