Mentzer Index Diagnostic Value in Predicting Thalassemia Diagnosis

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Abstract. Thalassemia diagnosis requires gen examination using Polymerase Chain Reaction (PCR) device or hemoglobin (Hb) electrophoresis using electrophoresis or High Performance Liquid Chromatography (HPLC) device which is only available in certain hospitals. Therefore, it is important to develop easily examined parameters which are useful to assess thalassemia carrier diagnosis. This study aims at figuring out the diagnostic value of Mentzer Index in predicting beta-thalassemia carrier compared to the results of HB electrophoresis examination. Data were taken from 37 anemia patients at Prof. Dr. Margono Soekarjo Regional Public Hospital in Purwokerto who underwent Hb electrophoresis examination using electrophoresis instrument and routine blood count. Furthermore, Mentzer Index was calculated, odds ratio (OR) was analyzed, and diagnostic values were compared to the results of Hb electrophoresis. Mentzer index OR value was 2.4 (0.5 - 11.5, CI95%). Mentzer Index sensitivity (Sn) was 0.36 with specificity (Sp) at 0.81, positive predictive value (PPV) at 0.44 and negative predictive value (NPV) at 0.75. Mentzer index could be used to predict the diagnosis of beta-thalassemia carrier.

Keywords: Mentzer Index, diagnostic value, thalassemia

1. Introduction

Thalassemia, a disorder caused by the decrease or absence of one or more globin chains' synthesis, is the most common genetic disorder in the world. WHO reported that 5% of the world's population is thalassemia carriers [1]. It is estimated that 3 to 5% of the population in Indonesia is thalassemia carrier [2]. Banyumas is the regency in Indonesia with a significant increase in the number of thalassemia patients. The Indonesian Thalassemia Foundation-Association of Thalassemia Patients' Parents or known as YTI-POPTI (*Yayasan Thalassemia Indonesia-Perhimpunan Orang Tua Pasien Thalassemia*) Banyumas branch has reported an increased number of thalassemia patients in Banyumas from 44 patients in 2008 to 65 patients in 2009. There was an increase of 53.85% in 2010 which made the number became 100 patients and another increase of 63% occurred in 2011 [3]. The number of patients exceeded 250 in 2012 [4]. Other researchers said the addition of cases was 3-7 patients per year equivalent to an increase of 4.76% -11.50% cases each year compared to the number of previous cases [4, 5]

Diagnosis of thalassemia is performed by doing preliminary, physical, and laboratory examinations. Ultimate laboratory tests include HbA2, HbF examinations, and DNA analysis to determine the type of mutation that occurs [6]. The gold standard for diagnosing thalassemia carriers is identified by an increase in HbA2 levels [7], but now DNA analysis is used to standardize thalassemia diagnosis based on the type of mutation that occurs.

Screening examination to recognize thalassemia carriers in Indonesia in general is based on the value of MCV <80 fl, MCH <27 pg, and Hb typing examination [8]. The

instrument used for Hb typing examination by hemoglobin electrophoresis using micro-column chromatography, High-Performance Liquid Chromatography (HPLC) as well as capillary iso-eletro focusing techniques is not widely available in Indonesia, especially for level I screening at regency/city hospitals [8].

It is necessary to develop a simple examination method that can be performed at the regency hospitals as well as district health centers. One of the examination parameters that can be used for thalassemia carrier screening test is Mentzer index. Mentzer index is an MCV/RBC ratio calculation in which patients with a value of <13 is diagnosed as thalassemia carriers while a value of >13 is found in patients with iron deficiency [9, 10].

2. Materials and Methods

This was a cross-sectional study conducted at Prof. Dr. Margono Soekarjo Regional Public Hospital in Purwokerto, Central Java. Population study was taken from 37 secondary data of all hemoglobin electrophoresis and routine blood count examination results of anemia patients from January to August, 2018. Hemoglobin electrophoresis data were obtained from the examination results using a capillary electrophoresis (SEBIA) instrument, while routine blood count data were obtained from examination results using Sysmex XN-1000 automatic hematologic analyzer. Thalassemia carrier diagnostic criteria were anemia with MCV <80 fl, MCH<27 pg, and HbA2 fraction> 3.5% [8,11,12]. Furthermore, a Mentzer Index (MCV / RBC) calculation with a value of <13 was diagnosed as a beta-thalassemia carrier. Statistical analysis was performed to calculate the odds ratio (OR) and diagnostic values in the form of sensitivity, specificity, positive predictive value and negative predictive value of Mentzer Index compared to hemoglobin electrophoresis.

3. Results and Discussion

This study revealed the result after hemoglobin electrophoresis examination upon 37 patients consisting of 24 females and 13 males 59.5% of the respondents were of children age. The result disclosed that there were 11 people (29.7% of the respondents) who were anemia patients and were diagnosed as carriers of beta-thalassemia based on criteria HbA2 >3.5%. 5 among the 11 experienced the raise in HbE value (19%-65%), thus they were diagnosed as HbE-beta-thalassemia. Research subjects' characteristics were presented in Table 1.

Table 1. Research Subjects' Characteristics

Variable	Category	Total	Percentage (%)
Sex	Male	13	35.1
	Female	24	64.9
Age	Children (<17 Years Old)	22	59.5
	Adult (≥17 Years Old)	15	40.5
HbA2	< 3.5%	26	70.3
	≥3.5%	11	29.7

Mean of Hb level, the number of erythrocytes, and MCV in the research subject were decreased with Mentzer mean index was 23.84. Mean of HbA2 was 3.15 and mean of HbF was 13%. The results of laboratory test were presented in Table 2.

Table 2. Blood Index Laboratory Test Result

Parameter	Minimum	Maximum	Average	SD	p*
Hb (g/dL)	2.80	12.70	7.82	2.44	.786
RBC (10^6/µL)	1.10	5.30	3.66	1.22	.008

MCV (fL)	47.40	107.00	70.69	13.33	.424
Mentzer Index	10.77	82.31	23.84	16.12	.000
HbA2 (%)	1.70	5.90	3.15	1.31	.000
HbF (%)	0.00	95.90	13.00	24.75	.000

*Normality test using Shapiro-Wilk

Table 3 disclosed the difference between mean of blood index in a beta-thalassemia carrier and non-carrier. It can be inferred that the results of HbA2 and HbF level were different on each group.

Table 3. Comparison of Blood Index in carrier and non-carrier of Beta-Thalassemia.

Parameter	**Average (SD)**		**Discrimination Test**
	carrier	**Non-carrier**	**(p)**
Hb (g/dL)	6.96 (2.57)	8.18 (2.35)	.168*
RBC (10^6/μL)	3.58 (1.39)	3.70 (1.16)	.857**
MCV (fL)	64,52 (15.40)	73.30 (11.72)	.066*
Mentzer Index	23.85 (20.93)	23.83 (14.11)	.421**
HbA2 (%)	5.08 (0.87)	2.38 (0.42)	.000**
HbF (%)	15.55 (16.60)	11.08 (26.98)	.040**

*Unpaired T-test, ** Mann-Whitney Test

The result of diagnostic Mentzer index showed low sensitivity as well as low Positive Predictive Value. On the contrary, specificity and Negative Predictive Value were quite high—as seen in Table 4. Result of odd-ratio was 2.4 (95% CI 0.5-11.5) with prevalence ratio of 1.76.

Table 4. Mentzer Index Diagnostic Value

Parameter	**Sensitivity**	**Specificity**	**NDP**	**NDN**
Mentzer Index	0.36	0.81	0.44	0.75

This research revealed that 29.7% of the 37 research subjects were diagnosed as beta thalassemia carrier. The percentage was far higher than the percentage of thalassemia carrier in Banyumas—which was 8% [5]. It was also higher than mean of thalassemia carrier in Indonesia which was 3-5% [2]. However, this result could not represent prevalence of thalassemia patients in Prof. Dr. Margono Soekarjo Regional Public Hospital. It was due to the data of examination regarding Hb electrophoresis during the period of January-August 2018. 45% of the 11 research subjects were diagnosed with HbE/β -thalassemia. The result was inclined with research conducted by [13] which found HbE/β^+ -thalassemia and HbE/β^0-thalassemia in 86 people and 30 people from 189 subjects respectively. It could be affected by characteristic of gen XmnI, BCL11A, and HBS1L-MYB found in thalassemia patients in Banyumas [13].

The majority of research subjects were females (64.9%). The result showed was not in accordance with the result of research conducted by [14,15] in which the majority of research subjects were males. However, the composition of research subject is similar with [16]. Thalassemia is a genetic disease that is not correlated to sex chromosome—therefore, both male and female have similar chance to be exposed to thalassemia [3].

HbA2 levels of research subjects who were beta-thalassemia carriers differed significantly from those of the non-carriers (5.08% vs. 2.38%). This study supported the classic results of blood indices examinations on thalassemia carriers, namely decrease of Hb, RBC, and MCV with HbA2 increase levels above 3.5% [17]. The results of previous

studies indicated that the value of HbA2> 3.5% was the best diagnostic test to determine beta-thalassemia carriers [18]. The results of this study were also in line with the results of other studies [5, 9, 10, 13, 14, 15]. Recently, there are more than 200 mutations in the β-globin gene that have been found. Differences in gene mutations will cause differences in the severity of clinical signs and symptoms in patients including blood indices, including Hb, RBC, and MCV levels. B^{+} mutations in globin chains will cause lower Hb, RBC, and MCV values lower than β^{0} mutations [17].

The sensitivity of Mentzer index resulted from this study was 0.36 with a specificity of 0.81 while the PPV was 0.44 and NPV 0.75. These results indicated that Mentzer index's ability to accurately diagnose beta-thalassemia was 0.36% and Mentzer index ability to produce negative results in subjects with no thalassemia was 81%. PPV of 0.44 meant that if the diagnostic test results of Mentzer index were positive then the probability of subjects to from beta-thalassemia was 44%. NPV 0.75 showed that if Mentzer index diagnostic test results were negative, then the probability of subjects not to suffer from beta-thalassemia was 75% [19,20,21] The results of this study are in accordance with Sharma's study that the Mentzer index sensitivity (60%) was lower than the specificity value (93.10%) as well as the PPV (60%) which was lower than the NPV (93.10%). The sensitivity and specificity of Mentzer index results of this study were lower than those of [22] with higher sensitivity values than specificity (94.5 vs 93.7) as well as higher NDP than NDN (95.9 vs 92.2). The low sensitivity of Mentzer index in this study might be due to the low number of RBCs (minimum amount of 1.1 million/µL) and the presence of high MCV values (107 fL). The Mentzer index is obtained from the division of MCV / RBC so that the higher the MCV and the lower the RBC caused Mentzer index value to be greater (> 13) so that the research subjects were not diagnosed as thalassemia carriers. Higher specificity than sensitivity value resulted from this study showed that Mentzer index had a higher diagnostic value in removing the possibility of beta-thalassemia carriers than in making a diagnosis. Odd ratio and prevalence ratio of Mentzer's index in this study were 2.4 and 1.76, respectively. These results had not been able to conclude that Mentzer index could be used to estimate the ratio of the incidence of thalassemia because in 95% CI, the value ranged from 0.5 to 11.5.

4. Conclusion

The results showed 29.7% of the study subjects were beta-thalassemia carriers, with 45% of them were HbE / β-thalassemia. Mentzer index diagnostic value resulted from this study were sensitivity 0.36, specificity 0.81, positive predictive value (PPV) 0.44 and negative predictive value (NPV) 0.75. These values meant that Mentzer index can be used to predict thalassemia carriers especially to remove the possibility of a diagnosis.

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