The Correlation of HbA2 Levels Against The Mentzer Index in Patients Diagnosed with Thalassemia in A Private Laboratory Around Central Java

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Abstract. Hemoglobin electrophoresis is the gold standard for thalassemia carriers screening. Hemoglobin electrophoresis sees an increase in HbA2, where thalassemia patients experience abnormalities in the composition of HbA2. The Mentzer index is a discrimination index formula with good sensitivity, specificity, accuracy, and positive predictive value in determining iron deficiency anemia or thalassemia. The aim of this study was to determine the correlation between HbA2 and the Menzer index in patients diagnosed with thalassemia at a private laboratory in Central Java. The study was conducted in January 2022-May 2023 with samples of 30 data from the results of Hb electrophoresis and routine complete blood counts in patients diagnosed with thalassemia in the CITO Laboratory. The results of the Rank Spearman correlation test concluded that there was a significant correlation with a negative coefficient pattern (r=-0.398), this means that the variables are said to be not unidirectional, meaning that if the HbA2 variable increases, the Menzter index variable will decrease, and vice versa. The Mentzer index is calculated from the results of a complete blood count, if the MCV divided by erythrocytes is less than 13, then it is declared as thalassemia. But if the result is greater than 13, then it is expressed as iron deficiency anemia. Patients with thalassemia found an increase in HbA2 levels by reading using the Mentzer index for thalassemia patients which is less than 13. The higher the HbA2 level, the Mentzer index value will be lower than 13.

Keywords: HbA2, Mentzer Index, Thalassemia.

1. Introduction

Hemoglobin electrophoresis is the gold standard in screening thalassemia carriers. Hemoglobin electrophoresis can see an increase in HbA2, where thalassemia sufferers experience abnormalities in the composition of HbA2. Normal HbA2 levels in the body are 2-3% [1]. The Mentzer index is one of the index discrimination formulas discovered by Mentzer in 1973. The Mentzer index formula is a calculation that has good sensitivity,

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specificity, accuracy and positive predictive value in determining the category of iron deficiency anemia or thalassemia [2]. The Mentzer Index is obtained by calculating the average cell volume or Mean Corpuscular Volume (MCV) divided by the number of erythrocytes. A Mentzer index calculation result of more than 13 is categorized as deficiency anemia, while a value of less than 13 is categorized as β thalassemia minor [3].

Data in Indonesia about thalassemia is one of the most frequently found genetic diseases among other genetic diseases. In some areas, the prevalence of carriers even reaches 10%. The large number of thalassemias in Indonesia occurs due to population migration and the mixing of populations from Southern China with a strong Mongoloid phenotype. The entire mixed-race population is spread across Java, Kalimantan, Sulawesi, Nias, Flores, Sumba and Sumatra [4]. As As reported on the Kementerian Kesehatan Indonesia website, 2022, based on data from the Yayasan Thalassemia Indonesia, yearly cases of thalassemia continue to increase [1]. Central Java Province occupies the top 3 positions in thalassemia cases, namely 920 in 2014, then in 2021 the prevalence increased to 7.22% [5].

Based on Susanto et al. (2019), research has been carried out to determine the correlation between the Menzer index and HbA2 levels in patients in Depok, West Java, with the results of a weak correlation and a negative pattern, if the HbA2 level increases, the Mentzer Index decreases. Since Central Java has the third highest number of cases in Indonesia, the second highest after West Java, the author is interested in researching the correlation between HbA2 levels and the Mentzer index in Central Java Province. This research aims to determine the correlation between HbA2 levels and the Mentzer Index in patients diagnosed with thalassemia in private laboratories in Central Java. In the future, this correlation can be used as a reference in carrying out thalassemia screening, so that cases of thalassemia in Indonesia can decrease [6].

2. Methods

The research method uses correlation analytics which is used to identify and analyze the correlation of Hemoglobin A2 levels with the Mentzer index in 30 patients diagnosed with thalassemia at the CITO Medical Laboratory of Semarang. The research was carried out in January 2022-May 2023 using a cross-sectional approach. Research respondents underwent complete peripheral blood assays, and then continued with hemoglobin electrophoresis assays.

The results of this assay were carried out by analysis using Microsoft Excel for the Mentzer index. Analysis continued with correlation tests using the Statistical Package for The Social Science (SPSS). The SPSS test for data normality uses Shapiro-Wilk and correlation uses the Spearman Rank test.

3. Result

3.1. Characteristics Respondents

Samples were collected from 30 patients diagnosed with thalassemia at the CITO Medical Laboratory of Semarang. The characteristics of the respondents are shown in Table 1.

Table 1. Gender Frequency Distribution of Thalassemia Patients

Characteristics	Frequency (n)	Percentage (%)
Gender		
Male	20	67
Female	10	33

Table 1 The frequency distribution of patients suffering from Thalassemia in the CITO Lab according to gender is mostly male patients with 20 patients (67%), while there are 10 female patients (33%).

Table 2. Distribution of HbA2 Levels and Mentzer Index in Thalassemia Patients

Variable	Mean	Median	SD	Minimal-Maximal
HbA2 Levels	3,88	3,50	1,29	2,00-6,30
Indeks Mentzer	10,16	10,67	1,35	8,00-11,90

The distribution of HbA2 levels in patients suffering from thalassemia in the CITO Lab was found to be an average of 3.88 with an SD of 1.29. The lowest HbA2 level is 2.00 and the highest is 6,30. The distribution of the Mentzer index of patients suffering from thalassemia in the CITO Lab was found to be an average of 10.16 with an SD of 1.35. The lowest Mentzer Index was 8.00 and the highest was 11.90.

Analysis of the correlation between HbA2 levels and the Mentzer index in patients with thalassemia in the CITO Laboratory was carried out using non-parametric analysis because the data were not normally distributed. The SPSS analysis program used is Spearman Rank correlation.

Table 3. Correlation of HbA2 Levels with the Mentzer Index

Correlation	n	R	*p-value*
HbA2 Levels vs Mentzer Index	30	-0.398	0.030

Table 3 shows that the correlation coefficient (r) value of HbA2 levels with the Mentzer index is -0.398. This means that between HbA2 levels and the Mentzer index in patients suffering from thalassemia in the CITO Lab has a weak correlation and has a negative

pattern, where if the HbA2 level value increases, the Mentzer index value will decrease, and vice versa. The statistical test results showed that there was no significant relationship between HbA2 levels and the Mentzer index in patients suffering from thalassemia at the CITO Lab (p-value >0.05).

4. Discussion

Based on table 1 and table 2, it is known that in this study the majority of thalassemia sufferers were male, namely 67%. This is in accordance with research conducted by Safitri, dkk (2015) that the frequency of female patients suffering from thalassemia is less than male patients [7]. Research conducted by Heldawati, et al (2023) also showed the same results, namely that the highest frequency of thalassemia sufferers was male (66.7%) [8]. The results of research from Ilmi, et al (2014) state that the frequency of male and female thalassemia sufferers is in the same proportion [9]. This is in accordance with the theory that thalassemia can be passed on to the next generation autosomally. Autosomal disorders are diseases carried by genes in autosomal or non-sex chromosomes [10]. Decreased thalassemia gene has a 25% chance of a child being born normal, a 50% chance of being a carrier of the trait and a 25% chance of being a sufferer. This possibility does not depend on gender, where the synthesis of the beta-globin polypeptide chain only takes place in cells of the erythroid series, even though the beta-globin gene also found in the chromosomes of other cells [11].

Based on table 3, the HbA2 level with the Mentzer index in this study has a significant correlation with a negative coefficient pattern (r=-0.398), this means that the variables are said to be unidirectional, meaning that if the HbA2 variable increases then the Mentzer index variable will decrease, and vice versa. This is in accordance with the theory that thalassemia sufferers will experience an increase in HbA2 levels when the Hb electrophoresis assay is carried out, while the Mentzer index will experience a decrease [12]. The Mentzer Index has 2 values, namely if the division result is more than 13, it is declared iron deficiency anemia, whereas if the division value is less than 13 it is declared thalassemia [2].

β-thalassemia minor (trait), is caused by the presence of 1 gene mutation and the other gene is normal. Patients who experience mild microcytic anemia also generally find HbA and HBA2 increased by a value of 4 to 7% of total hemoglobin [13]. There is a compensatory increase in HbF levels in the production of γ globulin chains, resulting in an increase in HbF of 2 to 8% in 50% of sufferers [6].

Thalassemia intermedia sufferers have a homozygous β-thalassemia phenotype, α and β-thalassemia defects or β-thalassemia with high HbF levels. Thalassemia minor has almost no visible symptoms at first. The clinical symptoms of β thalassemia are shown from birth, namely paleness, weakness, easy infection, difficulty eating, and impaired growth. Apart from that, in several cases splenomegaly and hepatomegaly were also found which resulted

in the stomach appearing distended [14]. Reported by the Indonesian Ministry of Health (2019) that thalassemia cannot be cured, one way to reduce the incidence of thalassemia is to carry out screening tests to support thalassemia and prevent marriage between thalassemia carriers [15].

One of the supporting assays for diagnosis and screening of thalassemia is hematology assay and peripheral blood imaging [16]. Supporting assays for the diagnosis and screening of thalassemia need to be further confirmed using hemoglobin electrophoresis [12]. This aims to determine the presence or absence of HbA and the increase in HbA2 and HbF. The Mentzer Index is one of the discrimination index formulas that was developed and used as a screening test for thalassemia traits to distinguish it from other microcytic hypochromic anemia, especially iron deficiency anemia, by calculating the average cell volume or mean corpuscular volume (MCV) divided by the number of erythrocytes [2].

NP Rembulan (2015) stated that the Mentzer index and RDW are most widely used for initial thalassemia screening. This results in the accuracy of iron deficiency anemia being the RDW index (88.14%) and the Mentzer index of 86.85%. Vehapoglu et al (2014) stated that the Mentzer index has a sensitivity of 98.7% and a specificity of 82.3% for the thalassemia screening test. The figures of 98.7% and 82.3% are the highest figures compared to other discrimination indices. Research conducted by Vehapoglu, et al (2014) also compared several indices used to differentiate iron deficiency anemia and β-thalassemia. The results are sequentially from those with high accuracy, namely the Mentzer index-Ehsani et al-RBC calculation index-Sirdah et al-RDWI index-Ricerca et al-Shine and Lal-MCHD index. Significant differences were found between the measurement results of each index and the gold standard for measuring HbA2 (p-value < 0.001) [17].

Based on research by Kristiana, et al (2018), the Mentzer index has a sensitivity and specificity for detecting iron deficiency anemia of 93.88% and 87.76% and for detecting beta thalassemia minor of 87.76% and 93.88%. The RDWI index has a sensitivity and specificity for detecting iron deficiency anemia of 89.90% and 83.67% and for detecting beta thalassemia minor of 83.67% and 89.90%. The Green and King index has a sensitivity and specificity for detecting iron deficiency anemia of 91.84% and 77.55% and for detecting beta thalassemia minor of 77.55% and 91.84% [18].

5. Conclusion

Correlation research on HbA2 levels and Mentzer index in Lab patients. CITO obtained the following conclusions: the average HbA2 level was 3.88, while the Mentzer index was 10.16. There is a significant correlation and a negative coefficient pattern ($r=-0.398$) between HbA2 and the Mentzer index in patients diagnosed with thalassemia in a private laboratory in Central Java. This means that the variables are said to be unidirectional, meaning that if the HbA2 variable increases then the Menzter index variable will decrease, and vice versa.

Suggestions for future researchers are to focus on beta-thalassemia patient respondents, both major and minor, and carry out further research using a genetic approach, namely using the Polymerase Chain Reaction (PCR) method and whole genome sequencing using the Next Generation Sequencing (NGS) method.

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References

1. Kementrian Kesehatan Republik Indonesia, Talasemia Penyakit Keturunan, Hindari dengan Deteksi Dini, 2022.
2. T.T. Sari, N. Aliza, S. Soedjatmiko, Indeks mentzer sebagai alat diagnosticik anemia defisiensi besi di sarana kesehatan dengan fasilitas terbatas: perbandinfan berbagai nilai cut off, Jurnal Sari Pediatri, 2019, vol. 21(3), p.1-8
3. BA, Ferdian, N, Rosdiana, B, Lubis B, Impact of iron therapy on Mentzer index and red cell distribution width index in primary school children with iron deficiency anemia, Medan: Pediatric Indonesia, 2009, vol. 49(4), p.195 – 196
4. R.A. Ganie, Thalassemia: Permasalahan dan Penanganannya, Medan: Universitas Sumatera Utara, 2005, p.2 – 3.
5. N.S.N. Handayani, A.A. Chyntia, Prevalensi thalassemia mayor di provinsi jawa tengah dan d.i. yogyakarta, Universitas Gadjah Mada, Yogyakarta, 2021.
6. H. Susanto, D. Susanti, Korelasi kadar HBA2 dengan indeks mentzer pada pasien thalassemia di rs hermina depok, Jurnal Ilmiah Analis Kesehatan, 2019, vol. 5(1), p-1-9
7. R. Safitri, J. Ernawaty, D. Karim, Hubungan kepatuhan transfusi dan konsumsi kelasi besi terhadap pertumbuhan anak dengan thalasemia, 2015, Jurnal Online Mahasiswa, vol. 2(2), p.1-10
8. F. Heldawati, R. Abdurrachim, M. Mahpolah, Kepatuhan minum obat kelasi besi, konsumsi sumber protein serta zat besi melalui pendampingan individu pada pasien anak thalassemia mayor (case series), Jurnal Skala Kesehatan Politeknik Kesehatan Banjarmasin, 2023, vol.14(1).
9. S. Ilmi, O, Hasanah, B. Bayhakki, Hubungan Jenis Kelamin dan Domisili dengan Pertumbuhan pada Anak dengan Thalasemia, Program Studi llmu Keperawatan: Universitas Riau, 2014.
10. L. Rujito, Talasemia: Genetik Dasar dan Pengelolaan Terkini untuk Mahasiswa Kedokteran dan Kesehatan. Purwokerto: Universitas Jenderal Soedirman, 2019.

11. D. Marnis, G. Indriati. F.A. Nauli, Hubungan tingkat pengetahuan ibu dengan kualitas hidup anak thalassemia, Jurnal Keperawatan Sriwijaya, 2018, vol. 5(2), p.1-7.
12. N.P.R. Rembulan, Indeks RDW dan Mentzer sebagai uji skrining diagnosis thalassemia, Medical Journal of Lampung University, 2015, vol. 4(7) 2015.
13. L.J. Wijaya, Y.M. Nency, M. Yetty, H. Farida, Faktor-faktor yang mempengaruhi prestasi belajar pasien thalassemia mayor anak. Jurnal Kedokteran Diponegoro, 2018, vol. 7(2).
14. A.D. Ardian, S, Andri, A, Tulus, Hubungan Kadar Hb dengan Ht pada Pasien Thalassemia. Semarang: Universitas Muhammadiyah Semarang, 2019
15. Kementerian Kesehatan Republik Indonesia., Thalassemia, Penyakit Berbiaya Tinggi ke-5 di Indonesia, 2019.
16. L. Rujito, Lantip. Talasemia: Genetik Dasar dan Pengelolaan Terkini untuk Mahasiswa Kedokteran dan Kesehatan. Purwokerto: Universitas Jenderal Soedirman, 2019.
17. A. Vehapoglu, G. Ozgurhan, A.D. Demir, S. Uzuner, M.A. Nursoy, A. Turkemen, A. Kacan, Hematological indices for differential diagnosis of β thalassemia trait and iron deficiency anemia, Anemia Journal, 2014, vol.1(7), p.1-7
18. R.H. Kristiana, N.S. Widyastiti, L. Setiawan, K. Edward, Uji sensitivitas dan spesifitas mentzer index, red distribution width index, dan green and king index terhadap diagnosis talasemia beta minor dan anemia defisiensi besi, Jurnal Kedokteran Diponegoto, 2018, vol.7(1).