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THE HEMOGLOBIN, RDW, AND MEAN CORPUSCULAR VALUES IN PATIENTS WITH BETA-THALASSEMIA/HEMOGLOBIN E DISEASE AND BETA-THALASSEMIA TRAIT

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ABSTRACT

Beta-thalassemia/hemoglobin E disease is a condition where there is double heterozygosity of beta-thalassemia trait and hemoglobin E trait. This produces a condition with more severe phenotypic appearance compared to beta thalassemia trait and hemoglobin E trait. Logically the Mean Corpuscular Values (MCV) of beta-thalassemia/hemoglobin E disease should also be worsened. The aim of this study was to assess the hemoglobin level, RDW, and MCV between beta-thalassemia/hemoglobin E disease and beta thalassemia trait. The researchers hereby studied eleven cases from two families who were detected to have beta-thalassemia/hemoglobin E disease. Family-1 with beta-thalassemia trait had MCV 68 fL and 65 fL, the MCH value was 21 pg and 20 pg, respectively. In Family-2, mother with beta-thalassemia trait, had MCV 60.2 fL and MCH 18. 8 pg. Daughters with beta-thalassemia/hemoglobin E disease from subjects 1 and 2 whose blood were taken repetitively during visits to the hematology clinic, had mean±SD of MCV 70.8±4.9 fL and Mean Corpuscular Hemoglobin (MCH) value 22.8±2.3 pg. They were significantly higher than the ones with beta-thalassemia trait ($p<0.05$). Moreover, there were found that the MCV from post-transfusion state were significantly higher than the pre-transfusion state ($p<0.001$). Based on the study, it could concluded that the MCV from subjects with beta-thalassemia/hemoglobin E disease were persistently higher than the beta-thalassemia trait. The role of blood transfusion in patients with beta-thalassemia/hemoglobin E disease seems to play a part in the result of a discrepancy in this matter.

Key words: Beta-thalassemia, hemoglobin E, discrepancy, MCV, RDW

INTRODUCTION

Hemoglobinopathy is the most common genetic disorder in Southeast Asia. Southeast Asia consists of ten countries: Indonesia, Malaysia, Singapura, Brunei Darussalam, Thailand, Myanmar, Kamboja, Laos, Vietnam, and Filipina. The ethnic origin of the people living in these countries is very heterogeneous. Malayo-Polynesian ethnic (Austronesian) live in Malaysia, Indonesia, Brunei, Philippines, and countries in the Pacific Ocean. Chinese and Indian tribes are spread in every country. Globally, thalassemia is the most common monogenic disorder, characterized by anemia caused by globin chain synthesis defects of adult hemoglobin.[1,2]

Thalassemia is an autosomal recessively inherited disease caused by the decrease or absent of globin chain synthesis. Based on the type of globin chain, thalassemia can be divided into α-, β-, γ-, δ-, δβ-, and εγδβ- thalassemia. The quantitative defect of globin chain can be accompanied by qualitative defects such as HbE, HbC, HbS, on beta-thalassemia and HbCS (Constant Spring) on alpha thalassemia. As an autosomal recessively inherited the disease, a person with heterozygote is state usually asymptomatic and does not need any therapy. Whereas individual with homozygote state and some type of heterozygote are suffered from thalassemia syndrome.[2,3]

Beta thalassemia carrier/trait is usually asymptomatic. The peripheral blood morphology show hypochromic and microcytic as well as an increase of HbA2 level. The hemoglobin type in heterozygote beta-thalassemia consist of 92-95% HbA, >3.8% HbA2, and variable HbF level (0.5 – 4%).[4]

Hemoglobin E is a variant of hemoglobin caused by a beta-globin gene mutation that results in glutamic acid substitution with lysine in codon 26 of beta-globin gene [codon 26 (GA)]. Hemoglobin E is the second most common globin abnormality after sickle cell hemoglobin. HbE is common in Southeast Asia, as much as 30-40% of all hemoglobin abnormality.[5,6]

Hemoglobin E trait is a heterozygote condition caused by marriage between one person with normal

hemoglobin and the other one with hemoglobin E variant producing offspring with ββE genotype. The hemoglobin electrophoresis show HbA, HbE, and HbA2.[5,6]

Beta-thalassemia/hemoglobin E disease occurs when the hemoglobin E trait gene combines with β^+ or β^0 thalassemia. This condition is commonly found in Southeast Asia, including Indonesia. Most of the patient show medium or severe condition with dependent on blood transfusion. The hemoglobin electrophoresis detects HbA, HbE, HbF, and HbA2 in beta+ thalassemia/hemoglobin E and HbE, HbF, and HbA2 in beta0 thalassemia/hemoglobin E.[5,6]

In Indonesia, the most common beta-globin gene mutations are IVS1-nt5 (G→C), IVS1-nt1 (G→T), codon 15/Cd15 ($TGG^{Triptofan}$→TAG^{stop}), codon 26 HbE ($GAG^{Glutamat}$→AAG^{Lysin}), and HbMalay/Codon 19 ($AAC^{Aspargin}$→AGC^{Serin}). Study from Semarang transfusion unit on a β thalassemic patient with routine transfusion the most common mutation is HbE/IVS1-nt5 (55.3%) followed by IVS1-nt5/IVS1-nt5 and HbE/Cd35 each as much as 13.2%.[7,8]

In Indonesia, according to data released by WHO, the percentage of pregnant female with beta-globin gene variant is 3,956% with A/β-thalassemia; 1,872% with AE, and 0.03% with another variant. Every year there are 192.842 pregnant females who are β-thalassemia carrier, 91.063 HbE carrier, and 1.459 carriers of another beta-globin gene variant. If both parents have the significant beta-globin gene variant, then each year Indonesia has risks as much as 10.256 birth with homozygous-β-thalassemia, 9.008 birth with β-thalassemia/HbE, and 2.640 birth with a combination of an undangerous variant. Every year in Indonesia there are 2.564 conceptions with homozygous β-thalassemia, 2.252 conceptions with β-thalassemia/HbE, and 660 conceptions with undangerous variant.[9]

The MC values: Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). They were first introduced by Wintrobe in 1929 to define size and hemoglobin concentration of red blood cells. Those three parameters usually termed as red cell indices and are very useful in determining the etiology of anemia. The red cells indices are calculated based on hemoglobin, hematocrit (packed cell volume), and red blood cell count. With the new technology, red cell indices can be automatically measured.[10]

The Red Cell Distribution Width (RDW) is the variation of red blood cells size. The size distribution of a population of cells is graphically represented by the red cells histogram (Price-Jones Curves).[10]

Mean corpuscular volume defines the red blood cells size in femtoliters (10-15/fL) or in cubic microns (μm3). The normal values are 87±7 fL. The MCV can be manually calculated using the formula:[10]

$$MCV = \frac{\text{hematocrit}}{\text{red blood cell count}} \times 10$$

The MCH quantifies the amount of hemoglobin in one red blood cell and, the normal value is 29 ± 2 picograms (pg) per cell. The MCH can be manually calculated using the formula.[10]

$$MCH = \frac{\text{hemoglobin}}{\text{red blood cell count}} \times 10$$

The RDW represents the coefficient of variation of the red blood cell volume distribution (size). The normal value is 13±1.5%.[10]

In everyday life, the majority of clinicians who work mostly in handling patients with hemoglobinopathy look first at the result of full blood count, which are the hemoglobin level, MCV as well as RDW. When those parameters were found to be abnormal and tend to side to the finding of A hemoglobinopathy, they required further investigation such as hemoglobin electrophoresis, High-Performance Liquid Chromatography (HPLC), and when confirmation was made, gene study was requested. So, the MCV and RDW is the first front gate for every clinician who has suspicion for investigating hemoglobinopathy.

This research finding in the hematology clinic, sometimes the MCV cannot give direct guidance and sometimes clinicians feel that the MCV are confusing. The MCV may give a potential benefit as a front gate for a guide of hemoglobinopathy. So, it was imperative that a study should be done to clarify if there was a discrepancy of MCV with a diagnosis of hemoglobinopathy, as well as the RDW in assisting the diagnosis.

METHODS

Two patients with beta-thalassemia/hemoglobin E disease were recruited in the study, and 45 blood samples were taken during the routine follow up examinations. For control comparisons, 5 samples were taken from beta-thalassemia trait patients. Three from their families and two from other beta-thalassemia trait cases. They were all cases from the Department of Pediatrics of the Medical Faculty of the University of North Sumatera/Adam Malik Hospital Medan. The beta-thalassemia/hemoglobin E

disease patients received regular blood transfusion for maintaining the hemoglobin level. Transfusion was given when the hemoglobin level was below 7 gr/dL or when the patients felt that the hemodynamic was disturb (such as weakness, shortness of breath, recurrent fever, and heart palpitation). All beta-thalassemia trait cases were not given a blood transfusion.

Six mL of venous blood was taken from the median vein. Three mL of blood was for the investigation of full blood count, and the other 3 mL of blood was for the investigation of genotyping study.

All beta-thalassemia/hemoglobin E disease and beta-thalassemia trait patients underwent full blood count examination every time they visit the clinic. The full blood count was investigated using Sysmex XN-1000. The hemoglobin level was investigated by the machine based on chemical analysis using lauryl sulfate, the MCV were automatically calculated by the machine based on the calculation of hemoglobin, hematocrit, and red blood cell count. The RDW was also automatically calculated based on the distribution width of the red blood cell which size and shape did not exit coefficient of variation of 15%, when the coefficient of variation was >15%, the RDW was considered as abnormally increased. The normal RDW was regarded as <15%.

The determination of beta-thalassemia trait and hemoglobin E trait, as well as beta-thalassemia /hemoglobin E disease, was carried out by means of capillary electrophoresis from Minicap Sebia where the machine uses a capillary tube to be filled up by EDTA blood and that blood in the capillary tube was run to the mini-chamber of an electrophoretic tank. The separation was automatically scanned by an in-machine densitometer and the result was compared with already made standard of normal hemoglobin, abnormal hemoglobin, and variant hemoglobin which was already provided inside the machine by the company.

The genotyping of the hemoglobin was also performed by ARMS-PCR and DNA sequencing done in Eijkman Institute For Molecular Biology in Jakarta. For this method, the DNA extraction was done by salting out method. After DNA was extracted, it was run into the ARMS PCR and DNA sequencing.

Statistical analysis was performed using a t-test for the sample with normal distribution and Mann-Whitney U Test for non-normal distribution. A correlation test was performed with Pearson for normal distribution and Spearman for non-normal distribution. This study was approved by the Health Research Ethical Committee of Universitas Sumatera Utara (No. 226/TGL/KEPK/ FK USU-RSUP HAM/2017).

RESULT AND DISCUSSION

From the hemoglobin electrophoresis investigation, it was found that there were two families which parents had one beta-thalassemia trait gene, and the other had hemoglobin E trait gene.

The father of Family-1 had beta-thalassemia trait gene and the genotype show IVS1nt5 (GC), while the mother had hemoglobin E genotype of codon 26 (GA). Together they have five children: one daughter

Table 1. Samples characteristics

	Age (years)	HbA (%)	HbA2 (%)	HbE (%)	HbF (%)
Family-1					
Father	50	95.1	4.9	-	-
Mother	46	73.6	3.5	22.9	-
Son-1	26	72.6	3.9	23.5	-
Son-2	24	96.8	3.2	-	-
Son-3	23	94.8	4.9	-	0.3
Daughter-1	12	97.1	2.9	-	-
Daughter-2	9	75.3	3.5	15.6	5.6
Family-2					
Father	32	70.7	3.2	26.1	-
Mother	32	93.5	6.2	-	0.3
Daughter	10	72.4	3.8	16.7	7.1
Son	8	97	3	-	-
Person-1	35	94.8	4.7	-	0.5
Person-2	26	95.3	4.7	-	-

with beta-thalassemia/hemoglobin E disease [IVS1nt5 (GC)/Codon 26 (GA)], one son with hemoglobin E trait (codon 26 GA/-), one son with beta-thalassemia trait (IVS1nt5 (GC)/-), one son and one daughter that normal.

Family-2: the father had hemoglobin E trait gene with the genotype show codon 26 (GA). The mother had a beta-thalassemia trait with the genotype show codon 41/42 (-TTCT). Together they had two children: one daughter with beta-thalassemia/hemoglobin E disease [Codon 41/42 (-TTCT)/Codon 26 (GA)] and one normal son. The result of the MCV can be seen in Table 2.

Table 2 shows that the Mean±SD of the MCV of patients with beta-thalassemia/hemoglobin E disease is significantly higher compared to beta-thalassemia trait.

The mean concentration hemoglobin of patients with beta-thalassemia/hemoglobin E disease was significantly higher compared to beta-thalassemia trait.

Figure 1 shows the mean differences between MCV and MCH in beta-thalassemia/hemoglobin E disease and beta thalassemia trait. The left Figure shows that the MCV level of beta-thalassemia/hemoglobin E is significantly higher than the beta-thalassemia trait with p-value 0.008 (using an independent t-test). The right Figure shows that the MCH level of beta-thalassemia/hemoglobin E is also significantly higher than the beta-thalassemia trait with p-value also 0.008 (using Mann-Whitney U Test).

Table 3 shows that the Mean±SD of the hemoglobin levels of patients with beta-thalassemia/hemoglobin E disease is significantly lower compared to beta-thalassemia trait. The RDW of patients with beta-thalassemia/hemoglobin E disease was significantly higher compared to beta-thalassemia trait.

Table 2. The MCV of patients with beta-thalassemia/hemoglobin E disease and beta-thalassemia trait

MCV	Beta- thalassemia/hemoglobin E disease	Beta-thalassemia trait
MCV (fL)	70.898 ± 4.897	64.640 ± 2.889
MCH (pg)	22.831 ± 2.287	19.720 ± 0.968

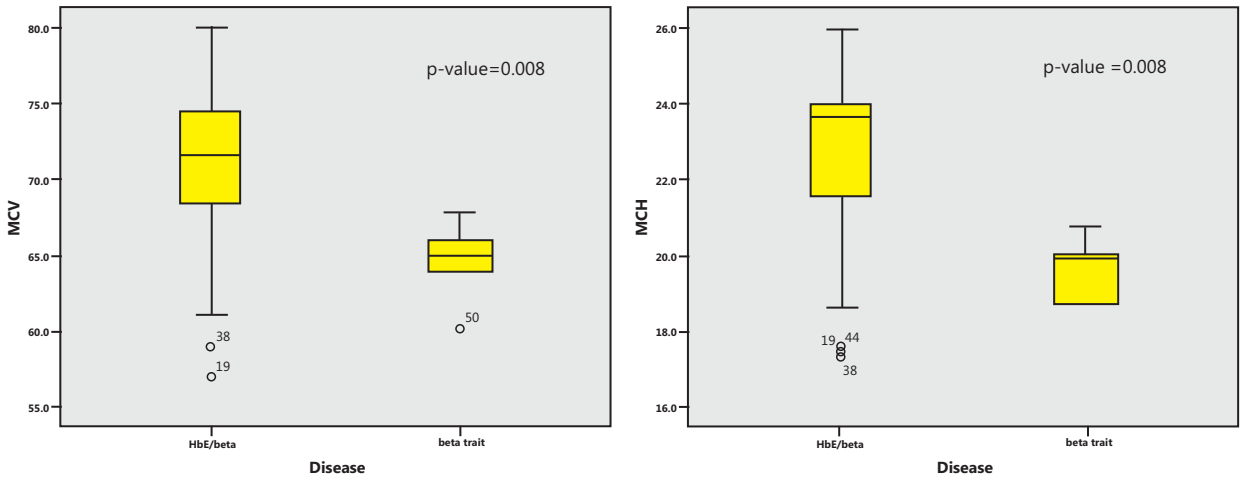

Figure 1. The differences between MCV between beta-thalassemia/hemoglobin E disease and beta-thalassemia trait

Table 3. The hemoglobin levels and RDW of patients with beta-thalassemia/hemoglobin E disease and beta-thalassemia trait

	Beta- thalassemia/hemoglobin E disease	Beta-thalassemia trait
Hemoglobin (gr/dL)	8.078±2.394	12.580±0.896
RDW (%)	29.340±3.978	16.160±0.422

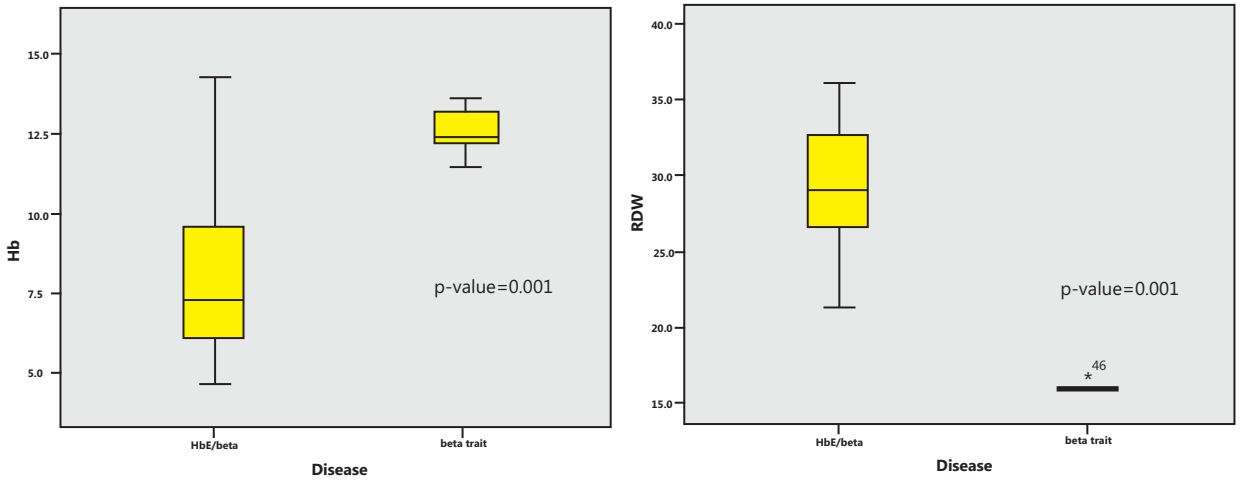

Figure 2. The differences in hemoglobin level and RDW between beta-thalassemia/hemoglobin E disease and beta-thalassemia trait

Table 4. The difference of hemoglobin level, MCV, MCH, and RDW between pre- and post-transfusion state in beta-thalassemia/hemoglobin E disease

	Pre-transfusion	Post-transfusion	p-value
Hemoglobin (gr/dL)	6.475 ± 0.939	10.465 ± 1.236	<0.001
MCV (fL)	68.757 ± 4.557	74.835 ± 2.873	<0.001
MCH (pg)	21.879 ± 2.305	24.494 ± 1.159	<0.001
RDW (%)	31.114 ± 3.125	26.235 ± 3.818	<0.001

Figure 2 show there are a significant difference of hemoglobin level and RDW between beta-thalassemia/hemoglobin E and beta-thalassemia trait with p-value 0.001 and <0.001 respectively (using Mann-Whitney U Test).

Moreover, divided the beta-thalassemia/hemoglobin E disease into pre-transfusion and post-transfusion state. This table show Mean±SD of hemoglobin level, MCV, MCH, and RDW in pre- and post-transfusion state. The result shows that there are significant differences between those four parameters between pre- and post-transfusion state. Beta-thalassemia/hemoglobin E disease is a condition where there is a combination of double heterozygosity of beta-thalassemia trait and hemoglobin E trait gene. This condition is more severe than the beta-thalassemia trait alone or hemoglobin E trait alone. Either in the phenotypic appearances such as clinical conditions, hemoglobin value, MCV, as well as RDW. Nonetheless, surprisingly the researchers found that the MCV of patients with beta-thalassemia/hemoglobin E disease had better result compared to beta-thalassemia trait alone. In this method, it was shown that the MCV and MCH were significantly higher in beta-thalassemia/hemoglobin E disease which logically a condition that was more severe and should produce a worse MCV.

Patients with newly diagnosed beta-thalassemia/hemoglobin E should not receive regular transfusion without long period observation of growth and development, quality of life, and spleen size. Transfusion should be administered if the hemoglobin level is <4 gr/dL and/or if the patient is suspected of having an acute intermittent infection, showing any problem suspected to be related to anemia. If a few transfusion have been administered in the acute situation, immediate commitment to a regular transfusion program should not be undertaken. During the initial assessment period, when the decision to administer regular transfusion is not decided yet, it is important to do full blood count examination every 2-3weeks.[11]

After 3-6 months of careful observation, a clinical and laboratory pattern should begin to emerge. Regular transfusion is not needed if the patients maintain reasonable appetite, level of energy, quality of life, develop good growth and development (height is better in depict growth pattern than weight), sexual maturation in parallel with bone age, and spleen size is stable (enlarging rate <3 cm/year).[11]

The regular transfusion should be considered if the hemoglobin level falls below 5 gr/dL or if

appetite, energy, growth, or developmental milestone is compromised, and if the spleen enlarging > 3 cm/year.[11]

In pediatric department of Adam Malik Hospital beta-thalassemia/hemoglobin E disease received regular transfusion if the steady-state hemoglobin level is below 7 gr/dL or if the patient show any hemodynamic instability sign such as weakness, shortness of breath, recurrent fever, and heart palpitation, this is to avoid retardation of growth (stunting). In this study, those two patients will come every 3-4 weeks to received blood transfusion or whenever their parents feel that their children become more parlor or if their children show weakness. The pediatrician usually gives packed red cell and the blood volume given to the patient is calculated based on the patient's body weight.

This study found that the MC level was significantly better in beta-thalassemia/hemoglobin E compared to beta-thalassemia trait. This fact was confusing because logically, beta-thalassemia/ hemoglobin E should had worse MC level. This might be due to regular transfusion they received. The data on our study showed that the MC level were significantly higher in post-transfusion state compared to the pre-transfusion state. From this finding, we can learn that children who come to the the physician for the first time with low hemoglobin level, a slight reduction of MC level, and increase RDW should be asked if they ever received blood transfusion recently. The iron deficiency anemia can be excluded. The physician should request full blood count, peripheral blood morphology, iron profile, and if needed hemoglobin electrophoresis. This examination is important in making a diagnosis before giving transfusion, because patient newly diagnosed with beta-thalassemia/hemoglobin E does not need a regular transfusion, instead they should be observed carefully to determine when they need a transfusion.

CONCLUSION AND SUGGESTION

This research found a discrepancy of MCV between beta-thalassemia/hemoglobin E disease with beta-thalassemia trait. This finding closely relates to the administration of blood transfusion. The researchers recommend that all patients with beta-thalassemia/hemoglobin E disease who show a discrepancy of the MC values should be investigated carefully with RDW as well as information about possible blood transfusion to avoid a mistake.

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