HbE Level and Red Cell Parameters in Heterozygous HbE With and Without α^0 -Thalassemia Trait

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Abstract We compared hemoglobin (Hb) E levels and red cell parameters between heterozygous HbE with and without α^0 -thalassemia trait and also determine their appropriated cut-off points for differentiating these two groups. High performance liquid chromatography analysis results and mean levels of red blood cell (RBC) parameters, including RBC count, total Hb, hematocrit, MCV, MCH and MCHC of heterozygous HbE with α^0 -thalassemia trait ($n = 183$) and without α^0 -thalassemia trait ($n = 1437$) were reviewed and compared. The α^0 -thalassemia status in these samples was detected by real-time PCR with SYBR Green1 and high resolution melting analysis. Mean levels of HbE, total Hb, MCV, MCH and MCHC of heterozygous HbE with α^0 -thalassemia trait were significantly lower than those of heterozygous HbE without α^0 -thalassemia trait ($P < 0.001$). In addition, HbE level at a cut-off value of $< 24\%$ was superior to MCV (< 80 fL) and MCH (< 27 pg) for differentiating the heterozygous HbE with and without α^0 -thalassemia trait with 100% sensitivity and 87.2% specificity. Despite certain limitations of this study like missing RDW and reticulocyte counts, and not testing for α^+ -thalassemia and Hb Constant Spring, we conclude that the HbE level at a cut-off point of $< 24\%$ is a useful marker for initial

discrimination between heterozygous HbE with and without α^0 -thalassemia trait.

Keywords α^0 -Thalassemia · Coinheritance · HbE · High performance liquid chromatography · Screening

Introduction

Hemoglobin (Hb) E [$\beta 26$ (B8) Glu→Lys, GAG > AAG; HBB: c.79G > A] is a β -hemoglobin variant caused by G > A substitution at codon 26 of β -globin chain that leads to a change of the amino acids from glutamic acid to lysine. This abnormal Hb occurs with a high frequency throughout many Asian countries. The overall prevalence of HbE in Thailand is about 20–30%, with the highest frequency of about 50% observed in northeastern Thai population [1, 2]. The β^E -globin chain is synthesized at a slightly reduced rate as compared to β -globin chain and hence has the phenotype of a mild form of β -thalassemia [3, 4]. The hematological parameters, including total Hb, hematocrit (Hct), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of the HbE trait, are usually observed in normal levels. Thus, the preliminary screening of HbE is done by using a dichlorophenol-indophenol (DCIP) test [5]. In addition, a hemoglobin analysis is used as the confirmation test, and it is performed by using high performance liquid chromatography (HPLC), low pressure liquid chromatography (LPLC) or capillary electrophoresis (CE) methods [6, 7]. A coinheritance of α^0 -thalassemia trait with heterozygous HbE results in a marked reduction of HbE level. Based on microcolumn chromatography method, HbE level less than 25.0% has been used for screening of double heterozygous α^0 -thalassemia/HbE [8]. However, the previous study showed

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that total Hb had a clear impact on the levels of HbE, MCV and MCH cut-off points used for screening of double heterozygous α^0 -thalassemia/HbE [9]. Thus, the aims of this study are to analyze the correlation between HbE levels and red cell parameters, including RBC count, total Hb, Hct, MCV, MCH and MCHC within two groups of heterozygous HbE with and without α^0 -thalassemia trait and also determine their appropriated cut-off points for initial discrimination of these two groups.

Materials and Methods

Blood Samples and Thalassemia Diagnosis

This report is a retrospective study. All HPLC analysis results from January to December 2016 were reviewed. Ethylenediamine tetraacetic acid (EDTA) blood samples were submitted to the Associated Medical Sciences Clinical Service Center (AMS-CSC), Chiang Mai University, Chiang Mai, Thailand for thalassemia diagnosis. Red cell parameters, including RBC count, total Hb, Hct, MCV, MCH and MCHC for the α^0 - and β -thalassemia screening were measured with an automated blood counter (ADVIA 2120i, Siemens, Munich, Germany). The screening for HbE was performed by using the DCIP test which positive reaction would show a cloudy appearance [5, 6]. While the confirmation for β -thalassemia and HbE diagnostic test was performed by using the HPLC (VARIANT™ β -thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). In addition, the molecular diagnosis for α^0 -thalassemia (SEA and Thai type deletions) was detected by using the real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis as previously described [10].

Statistical Analysis

The HPLC data (HbA, HbF and HbE) and red cell parameters (RBC count, total Hb, Hct, MCV, MCH and MCHC) were presented by mean and standard deviation (SD). Statistical analyses were performed using SPSS software package (Statistical Package for the Social Sciences 11.0, Chicago, IL, USA). These data were also compared between the two groups of heterozygous HbE with and without α^0 -thalassemia trait by using Compare Means with Independent Samples *t* test. In addition, the correlations between HbE levels and red cell parameters within these two groups were analyzed by using Pearson Correlation. The *P* < 0.05 was considered statistically significant.

Results

From January to December 2016, the 10,573 blood samples were submitted to the AMS-CSC, Chiang Mai University, Chiang Mai, Thailand for the thalassemia diagnosis. The 1943 (18.4%) samples were diagnosed as heterozygous HbE and 235 (2.2%) were double heterozygous α^0 -thalassemia SEA type deletion/HbE. However, the red cell parameters including RBC count, total Hb, Hct, MCV, MCH and MCHC are available in 1437 heterozygous HbE and 183 double heterozygous α^0 -thalassemia SEA type deletion/HbE. Mean levels of HbE, total Hb, MCV, MCH and MCHC of heterozygous HbE with α^0 -thalassemia SEA type deletion trait were significantly lower than those of heterozygous HbE without α^0 -thalassemia trait. Whereas, the mean levels of HbA and RBC count of the former was significantly higher than those of the latter. However, the retention times of HbE among the two groups were not significantly different (Table 1).

The correlations between the HbE levels and red cell parameters within two groups of heterozygous HbE with and without α^0 -thalassemia trait are shown in Table 2. A negative correlation between HbE levels and RBC count was observed in the group of heterozygous HbE with α^0 -thalassemia trait while a positive correlation was found in the group of heterozygous HbE without α^0 -thalassemia trait. In addition, the positive correlations between HbE levels and total Hb, Hct, MCV and MCH were found in both groups. Moreover, the positive correlation between HbE levels and MCHC was found in only the heterozygous HbE with α^0 -thalassemia trait.

Discussion

The heterozygous HbE with α^0 -thalassemia trait had lower levels of HbE, total Hb, MCV, MCH and MCHC than the heterozygous HbE without α^0 -thalassemia trait. In addition, all of 183 (100%) heterozygous HbE with α^0 -thalassemia trait had HbE levels lower than 24.0%, MCV < 80 fL and MCH < 27 pg. At these cut-off points, these red cell parameters had 100% sensitivity for screening of α^0 -thalassemia trait in heterozygous HbE. However, the microcytic hypochromic anemia with MCV < 80.0 fL and MCH < 27.0 pg are also found in iron deficiency anemia, anemia of chronic disease, lead toxicity and sideroblastic anemia [11]. In current study, the 1260 (87.7%) and 1321 (91.9%) of 1437 heterozygous HbE without α^0 -thalassemia trait had low levels of MCV (< 80.0 fL) and MCH (< 27.0 pg), respectively. Thus, the specificity of MCV and MCH for differentiating the heterozygous HbE with and without α^0 -thalassemia trait was 12.3 and 8.1%,

Table 1 Red cell parameters of heterozygous HbE with and without α^0 -thalassemia trait

Red cell parameters	HbE with α^0 -thalassemia (n = 183)	HbE without α^0 -thalassemia (n = 1437)	P values
HbA (%)	74.2 $\pm$ 6.0 (61.0–86.8)	65.1 $\pm$ 5.8 (50.5–84.8)	< 0.001
HbF (%)	0.9 $\pm$ 1.3 (0.0–9.3)	0.8 $\pm$ 1.0 (0.0–9.3)	0.33
*HbE (%)	18.8 $\pm$ 2.0 (13.2–23.0)	26.8 $\pm$ 2.4 (16.2–37.4)	< 0.001
Retention time of HbE (min)	3.63 $\pm$ 0.01 (3.61–3.66)	3.64 $\pm$ 0.01 (3.62–3.66)	0.08
RBC count ($\times 10^{12}/L$)	5.8 $\pm$ 0.7 (3.8–7.7)	5.1 $\pm$ 0.6 (2.8–6.8)	< 0.001
Total Hb (g/L)	120 $\pm$ 17 (68–157)	126 $\pm$ 17 (66–174)	< 0.001
Hct (L/L)	0.39 $\pm$ 0.05 (0.23–0.49)	0.39 $\pm$ 0.05 (0.21–0.53)	0.98
MCV (fL)	66.8 $\pm$ 6.9 (42.0–79.0)	76.6 $\pm$ 4.3 (50.0–95.0)	< 0.001
MCH (pg)	20.8 $\pm$ 2.5 (13.7–25.9)	24.9 $\pm$ 1.8 (14.5–34.7)	< 0.001
MCHC (g/L)	310 $\pm$ 13 (267–339)	325 $\pm$ 11 (253–433)	< 0.001

The data are expressed as mean $\pm$ SD (ranges)

*HbA₂ and HbE are co-eluted on HPLC however a very minor fraction of the HbE level is made up of HbA₂ in heterozygous HbE

Table 2 The correlations between the HbE levels and red cell parameters within two groups of heterozygous HbE with and without α^0 -thalassemia trait

Correlations between	HbE with α^0 -thalassemia trait (n = 183)		HbE without α^0 -thalassemia trait (n = 1437)	
	Correlation coefficient (r)	P values	Correlation coefficient (r)	P values
HbE and RBC count	– 0.283	< 0.001	0.189	< 0.001
HbE and total Hb	0.440	< 0.001	0.217	< 0.001
HbE and Hct	0.337	< 0.001	0.228	< 0.001
HbE and MCV	0.718	< 0.001	0.097	< 0.001
HbE and MCH	0.739	< 0.001	0.073	0.006
HbE and MCHC	0.453	< 0.001	0.014	0.60

respectively. Whereas, only 184 (12.8%) of 1437 heterozygous HbE without α^0 -thalassemia trait had a low level of HbE (< 24.0%). Therefore, the HbE level at this cut-off point (< 24.0%) had 87.2% sensitivity and it was superior to the MCV and MCH for differentiating the heterozygous HbE with and without α^0 -thalassemia trait. Contrary with the previous studies showed that the combination of cut-off points of HbE, MCV and MCH clearly increased the efficiency in detecting the double heterozygous α^0 -thalassemia SEA type deletion/HbE [9, 12]. The contrary results could be due to the different cut-off values of these red cell parameters, particularly when a higher cut-off value of HbE (> 25.0%) was used for screening of α^0 -thalassemia trait, the consideration in combination with MCV and MCH is compulsory.

The previous study showed that the total Hb had a clear impact on the levels of HbE, MCV, and MCH cut-off points used for screening of the double heterozygous α^0 -thalassemia SEA type deletion/HbE [9]. However, in present study, there were no heterozygous HbE with α^0 -thalassemia trait samples which had HbE level higher than

24.0%. The positive correlation between total Hb and HbE levels was also found in these samples. Furthermore, 90 of 183 (49.2%) heterozygous HbE with α^0 -thalassemia trait had total Hb equal or higher than 12 g/dL while the HbE $\geq 24.0\%$ was observed in 877 of 1437 (61.0%) heterozygous HbE without α^0 -thalassemia trait which had total Hb equal or higher than 12 g/dL.

Although the previous study showed that the levels of HbE, total Hb, MCV, and MCH of double heterozygous α^+ -thalassemia/HbE traits were similar to those of HbE traits [9], we cannot rule out the homozygous α^+ -thalassemia or compound heterozygous α^+ -thalassemia and Hb Constant Spring (HBA2: c.427T > C) or Hb Paksé (HBA2: c.429A > T) in the heterozygous HbE without α^0 -thalassemia trait samples. Because the molecular analysis for detection of α^+ -thalassemia (3.7 and 4.2 kb deletions), Hb Constant Spring and Hb Paksé was not routinely performed in our laboratory. In addition, α^+ -thalassemia usually has a totally silent phenotype [13] and a prenatal diagnosis is not compulsory in a couple who has a fetus with risk for deletional and non-deletional HbH diseases.

The limitation of this study was the missing data of RDW, reticulocyte, white blood cell differential count and platelet count. Thus, the correlation between HbE and these hematological parameters within two groups of heterozygous HbE with and without α^0 -thalassemia trait cannot be analyzed. The previous studies reported that iron deficiency and megaloblastic anemia (MCV > 110 fL) affected the HbA₂ levels. However, the changes of HbA₂ levels did not affect the laboratory interpretation results [14, 15]. These effects may be occurred in HbE trait with megaloblastic anemia or iron deficiency anemia. Since the megaloblastic anemia with MCV > 110 was not found in the current study and the iron study is not done routinely in the AMS-CSC, the effect of megaloblastic anemia or iron deficiency anemia on the levels of HbE needs to be further studied.

In conclusion, our results indicated that decreasing in α -globin chain in double heterozygous α^0 -thalassemia/HbE affected HbE level. However, the positive correlations between HbE levels and total Hb, Hct, MCV and MCH were found in the both groups of heterozygous HbE with and without α^0 -thalassemia trait. Whereas, all of heterozygous HbE with α^0 -thalassemia trait samples had HbE level less than 24.0% thus this cut-off point is a useful marker for initial discrimination between heterozygous HbE with and without α^0 -thalassemia trait.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest.

Ethical Standard This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.

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